TESIS DOCTORAL

Soil quality and factors of organic matter stabilization in agricultural soils recently converted to irrigation in the Ebro Valley

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que la presente memoria de Tesis Doctoral titulada "Soil quality and factors of organic matter stabilization in agricultural soils recently converted to irrigation in the Ebro Valley" elaborada por D. Marcos Apesteguia Barberena, ha sido realizada bajo nuestra dirección, y que cumple las condiciones exigidas por la legislación vigente **para optar al grado de Doctor.**

Y para que así conste, firma la presente en Pamplona a 28 de Septiembre de 2015.

Dr. Iñigo Virto Quecedo Dr. Luis Orcaray Echeverria

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RESUMEN

La dinámica de estabilización de la materia orgánica en el suelo desempeña un papel fundamental en la calidad del suelo y en el ciclo global del carbono. En el contexto de suelos agrícolas calcáreos de clima mediterráneo semiárido en el que se desarrolla la presente tesis, esta dinámica puede verse modificada por diversos factores tanto edafoclimáticos como derivados del manejo agrícola. El cambio de uso de suelo de secano a regadío es considerado de gran importancia en este contexto, debido tanto a la intensificación de la transformación a regadío de suelo agrícola en los últimos años, en la zona del valle del Ebro, como al importante efecto que puede tener sobre la dinámica de la materia orgánica. Sin embargo, sobre este último punto el conocimiento actual es escaso.
Con el objetivo de profundizar en estos aspectos, la tesis se estructura en varios capítulos, que abarcan el estudio de los factores que condicionan la dinámica de la materia orgánica, y su efecto sobre la calidad del suelo y la estabilización de carbono en suelos agrícolas del valle del Ebro.

En primer lugar, dentro del proyecto "Calidad del suelo, dinámica de la materia orgánica y secuestro de carbono en los regadíos de Navarra", financiado por el Instituto Nacional de Investigaciones y Tecnologías Agrarias y Alimentarias (INIA), se desarrollo el monitoreo a nivel regional del efecto de la transformación a regadío sobre la estabilización de carbono orgánico en el suelo y los principales factores que lo condicionan (Capítulo I, caso de estudio). Para ello, se seleccionaron parcelas en tres zonas de Navarra, en regadíos de diferente antigüedad (5, 7 y 9 años) y características edafoclimáticas. Los datos obtenidos en cuanto al contenido de materia orgánica (C orgánico) y de su fracción más fácilmente biodegradable (C en la materia orgánica particulada) mostraron que el contenido de materia orgánica en las tres zonas estudiadas fue equivalente a zonas más húmedas. Además, la estimación realizada en una de las zonas de estudio a partir de la parcela contigua en secano, arrojó un secuestro de 19.4 Mg C ha^{-1} durante 7 años en los primeros 20 cm del suelo (horizonte labrado). Por otro lado el estudio de diversos factores (edáficos, climáticos y de manejo agrícola), evidenció que las propiedades del suelo fueron el principal factor condicionante del almacenamiento de materia orgánica tras la puesta en regadío.

Paralelamente (capítulo II) se desarrolló un estudio en profundidad y a nivel de parcela experimental de la dinámica de la materia orgánica en el suelo tras su transformación a regadío. El estudio, posibilitó un seguimiento de la dinámica de la materia orgánica durante dos años desde el momento inicial del cambio de manejo. Para ello, se

establecieron cuatro tratamientos diferenciados en la misma parcela; trigo en secano, trigo en regadío, maíz en secano y maíz en regadío. En ellos se controló tanto las entradas de materia orgánica al suelo (restos de cultivo) como la incorporación al suelo y a los distintos niveles de su estructura (agregados) del carbono orgánico incorporado. El seguimiento de este carbono orgánico se realizó a través del análisis de su firma isotópica, basándonos en la diferencia existente entre la vegetación hasta ahora presente C3 (trigo) y la vegetación C4 (maíz), introducida con el regadío. A pesar de no encontrarse diferencias ni en las entradas de carbono orgánico ni en el contenido total de carbono orgánico en el suelo entre secano y regadío, el seguimiento de la firma isotópica del carbono en las parcelas con maíz (secano y regadío) permitió observar diferencias en la dinámica de incorporación de la materia orgánica. La proporción de carbono orgánico proveniente de maíz fue casi tres veces mayor en regadío que en secano y su incorporación en agregados de menor tamaño fue mayor también, evidenciando una aceleración del ciclo de incorporación de la materia orgánica en condiciones de regadío.

Las observaciones realizadas en estos primeros estudios evidenciaron la necesidad de profundizar en el papel de los factores edáficos sobre la estabilización de la materia orgánica. Concretamente, el efecto de los carbonatos es de especial interés en nuestra región debido a la abundancia de suelos calcáreos. La influencia de los carbonatos en el estudio de la dinámica de estabilización de la materia orgánica se planteó desde dos puntos de vista diferentes (capítulos III y IV).

El capítulo III se desarrollo a partir de la hipótesis de que la presencia de carbonatos en la matriz mineral del suelo favorece la estabilización de la materia orgánica a través de limitaciones físicas a su descomposición. Con este objetivo se estudio el nivel de protección de la materia orgánica en dos suelos adyacentes y bajo el mismo manejo agrícola. El primero de ellos (Typic calcixerept) con un alto contenido en carbonatos y el segundo (Calcic Haploxerept) sin presencia de carbonatos. En ambos suelos se midió su contenido de carbono orgánico y se analizó su porosidad. La disponibilidad biológica de la materia orgánica en el suelo se estudió mediante el CO_2 respirado durante 30 días de incubación de las muestras de suelo. Con este fin se incubaron tanto muestras de suelo completo como muestras de los agregados de 2 a 5 mm. Además también se incubaron, en cada caso, muestras intactas y desagregadas con el objetivo de estudiar el efecto de la protección física. Los resultados mostraron, efectivamente, un mayor contenido de carbono orgánico en el suelo con carbonatos, mostrando además una tasa de respiración menor durante las incubaciones que el suelo sin carbonatos. Sin embargo, las escasas diferencias mostradas entre los suelos en sus características físicas, así como el mayor aumento de la respiración de las muestras de suelo sin carbonatos tras ser desagregadas, evidenciaron que una mayor protección física no es la causa de la mayor estabilidad de la materia orgánica en el suelo con carbonatos.

En el capítulo IV se abordó la problemática del análisis del carbono orgánico en suelos con carbonatos (presencia de carbono inorgánico). Dos métodos han sido utilizados tradicionalmente, (1) oxidación húmeda y (2) combustión total, en el que el carbono orgánico se puede medir tanto directamente tras eliminar el carbono inorgánico mediante un pretratamiento ácido, como indirectamente, substrayendo al carbono total el carbono inorgánico obtenido mediante un calcímetro. Ambos métodos han demostrado tener limitaciones metodológicas, por ello en el presente estudio se ha evaluado la adecuación de un método alternativo, el análisis térmico, que posibilita la cuantificación simultánea del carbono orgánico e inorgánico. Además, el análisis térmico de muestras tras ser sometidas a fumigación ácida permitió evaluar los efectos de este método utilizado como pretratamiento para la eliminación de carbonatos. Los resultados obtenidos mediante análisis térmico mostraron una buena correlación con los métodos de medida habituales, por lo que su uso para la medición simultánea de carbono orgánico e inorgánico resultó viable. A pesar de ello el método mostró algunas desventajas, como la escasa disponibilidad actual de equipos capaces de realizar estas medidas, y la necesidad del análisis individual de las muestras que ralentiza el proceso. Finalmente, se observó que la fumigación ácida afecta a la labilidad térmica de la materia orgánica, además una eliminación incompleta de los carbonatos fue detectada. Por lo tanto, este método debe ser manejado cuidadosamente en la cuantificación de carbono orgánico, y debe evitarse cuando las muestras vayan a utilizarse para posteriores análisis de calidad de la materia orgánica.

Por último, el capítulo V recoge el trabajo realizado con el objetivo de evaluar los indicadores de calidad de suelo más adecuados para suelos en regadío en nuestra región. Además, se testó la adecuación a nuestras condiciones agroclimáticas de un índice de calidad de suelo reconocido internacionalmente, “soil management assesment framework” (SMAF). La selección de indicadores de calidad de suelo, se realizó mediante el análisis de componentes principales y a partir de aquellos indicadores previamente seleccionados en condiciones de secano en la región. Los resultados mostraron un cambio de dichos indicadores tras la transformación a regadío. Lo que confirmó la necesidad de una reevaluación de los indicadores de calidad de suelo cuando las condiciones del agrosistema son modificadas. Además, los resultados obtenidos mediante SMAF permitieron comprobar su sensibilidad a los cambios de calidad de suelo en nuestras condiciones. A pesar de ello, un análisis detallado de los algoritmos de puntuación que utiliza SMAF para adaptarlos mejor a nuestras condiciones se consideró necesario.

En resumen, el trabajo realizado en esta tesis ha permitido avanzar en el conocimiento de los mecanismos de estabilización de la materia orgánica en suelos agrícolas semiáridos mediterráneos, así como de los factores tanto edáficos como de manejo agrícola que los condicionan. En especial

se ha profundizado en el efecto que tiene sobre ellos la transformación a regadío y su influencia sobre la calidad del suelo y la estabilización en él de carbono orgánico.
Este conocimiento sobre el ciclo de la materia orgánica y su relación con la calidad del sueo, ha permitido recabar datos sobre las potencialidades del regadío y otros manejos agrícolas, como el no laboreo, para el estabilización del C orgánico en suelos agrícolas de la región. Así mismo, se ha puesto en evidencia la necesidad de desarrollar herramientas específicas de evaluación de la calidad del suelo en el contexto de suelos de zonas semiáridas transformadas o en fase de transformación del secano al regadío.

SUMMARY

The organic matter stabilization dynamic in soil has an essential role in soil quality and in the carbon global cycle. In agricultural calcareous soil within the semiarid Mediterranean area where this thesis has been developed, this dynamic could be influenced by several soil, climate or management factors. In this context the introduction of irrigation is considered of great importance, due to the expansion of irrigation in the last years along the Ebro valley area, and the important effect it may have over organic matter dynamics. Even though, scarce knowledge currently exists about its effect. The objective of this work was to gain knowledge on these aspects. That for, this thesis is divided into several chapters, and they are devoted to the study of the factors which influence organic matter dynamics and its effect on soil quality, and organic carbon stabilization in agricultural soils of the Ebro valley.

Firstly, within the project “Soil quality, organic matter dynamic and carbon sequestration in irrigated land in Navarra” fund by the National Institute of agricultural research and technology (INIA), a regional monitoring of the effect of irrigation on organic carbon stabilization in soil and the main influencing factors was developed (Chapter I, case study). That for, agricultural plots in three areas of Navarra where selected, covering different soil and climate characteristics and different ages under irrigation (5, 7, 9). The obtained data about Soil organic matter (organic C) and particulate organic matter (C-POM) showed that the organic matter content in the three studied areas was equivalent to the content in more humid areas. Moreover, 7 years after irrigation setup the estimated organic carbon increase calculated in one of the plots, taking as reference an adjacent non irrigated plot, resulted in 19.4 Mg C ha^{-1} in the first 0-20 cm (tilled depth). Additionally, the study of several stabilization factors (soil type, climate, and agricultural management) evidenced that soil properties were the main influencing factor of soil organic matter increase after irrigation setup.

At the same time (Chapter II) an in deep study of soil organic matter dynamics after irrigation setup was developed in an experimental plot. This study made possible a detailed monitoring of soil organic matter dynamics since the moment of irrigation setup in the plot. That for, the experimental plot was divided in 4 different treatments; rainfed wheat, irrigated wheat, rainfed maize and irrigated maize. Organic matter inputs (crops biomass) and its incorporation to the soil and the different soil aggregation levels were measured in the 4 treatments. Organic carbon incorporation to the soil was monitored trough isotopic analysis, based on the differences in the isotopic signature of carbon of C3 plants (wheat)

traditionally present in the plot, and C4 plants (maize) introduced together with irrigation. No differences were found in organic carbon inputs and the soil organic carbon stocks between irrigated and dryland plots two years after irrigation setup. However, the isotopic analysis of organic carbon in maize plots showed differences in organic matter incorporation dynamic. The proportion of organic carbon from maize already present in the irrigated soil was near three times the proportion observed in dryland. Moreover, its incorporation to smaller aggregates was also greater, indicating an accelerated organic matter incorporation cycle in irrigated conditions.

The observations done in these initial studies evidenced the need of a deeper study of the role of soil properties as factors of organic matter stabilization. Specially, the influence of carbonates is important in our region due to the abundance of calcareous soils. The influence of carbonates in the soil organic matter stabilization dynamics study was considered from two different points of view (Chapter III and IV).

Chapter III was developed based on the hypothesis that the presence of carbonates in the soil mineral matrix may favor organic matter stabilization trough physical limitations to mineralization. With that aim soil organic protection level was studied in two adjacent soils under the same agricultural management. One of the soils (Typic calcixerept) had high carbonates content and the second one (Calcic Haploxerept) had no carbonates. Soil organic carbon content and porosity were measured in both soils. Organic matter availability for decomposers was studied trough respired CO_2 in a 30 days incubation of soil samples. That for, whole soil samples and 2-5 mm aggregates samples were incubated. Additionally, disaggregated samples from whole soil and aggregates were also incubated in order to study the effect of physical protection. Results, showed a greater soil organic carbon content in the carbonated soil, and smaller respiration rate during the incubations than non carbonated soil. However, the scarce differences in physical properties between soils, together with a greater increase of respiration in non carbonated disaggregated samples, indicated that a greater physical protection is not the cause of greater stability of soil organic matter in the carbonated soil.

In chapter IV soil organic carbon analytical issues in carbonated soils were addressed. Traditionally, two methods have been used, (1) wet oxidation and (2) dry combustion, in which organic carbon is measured either directly after inorganic carbon removal trough and acid pretreatment, or indirectly by subtracting to total carbon the inorganic carbon measured in a calcimeter. Both methods have demonstrated some analytical limitations; therefore the study developed in chapter IV evaluated an alternative method, ramped combustion, which makes it possible to analyze simultaneously organic and inorganic carbon. Moreover, thermal analysis of the samples after acid fumigation was

carried out for the evaluation of the effect of this acid pretreatment over soil organic matter. Results obtained trough thermal analysis showed high correlations with the traditional methods applied, proving to be an adequate method for simultaneous measurement of organic and inorganic carbon. Nevertheless, this method showed some disadvantages, such as scarce availability of the equipment, and the need of individual analysis of the samples. Finally, was observed that acid fumigation affects organic matter thermal lability, and uncompleted decarbonation of the samples was detected. Therefore, acid fumigation should be managed carefully in soil organic carbon determination and must be avoided when samples will be used in subsequent organic matter quality analysis.

Chapter V include the work carried out with the aim of determine the most sensible soil quality indicators for irrigated agricultural soils in our region. In addition, the adequacy to our agroclimatic conditions of an international recognized soil quality index "soil management assessment framework" (SMAF) was tested. The soil quality indicators selection was made based on the previously selected indicators in dryland conditions and using principal component analysis. Results showed a change in these indicators after irrigation setup. Such a change confirmed the need of reevaluate soil quality indicators when the agroecosystem characteristics are modified. The results obtained by the use of SMAF revealed that this framework resulted sensitive to the observed differences in soil quality among soil managements, and were therefore adequate to evaluate soil quality differences among them. Even though, a detailed analysis of some of the interpretation algorithms seems necessary to adapt the SMAF to the local agroclimatic conditions.

In summary, the work developed in this thesis constitutes an advance in the present knowledge about soil organic matter stabilization mechanisms in Mediterranean semiarid agricultural soils. As well as in the factors that influence these mechanisms, especially we have gone into detail on the effect of irrigation setup, and its influence over soil quality and the stabilization of soil organic carbon.

This knowledge gain about organic matter dynamics and its relation to soil quality allows for determining the potentialities of irrigated land and soil management strategies such as no-tillage, for organic C stabilization in agricultural land in the region. Also, it has been shown that there is a need for developing or adapting tools for soil quality evaluation in semi-arid areas transformed from rainfed to irrigated agriculture.

Chapter I

Introducción

Objetivos de la tesis

CHAPTER I

Introducción y objetivos de la tesis

Contenidos

INTRODUCCION

1. La materia orgánica del suelo

El carbono orgánico del suelo en el ciclo corto del C. El ciclo global del carbono está definido por sus reservorios en cinco compartimentos ambientales (atmósfera, geosfera, pedosfera, hidrosfera y biosfera), y por los flujos de este elemento que se producen entre ellos. Estos flujos ocurren a escalas muy diferentes, y obedecen por lo tanto a procesos controlados por factores que actúan a escalas temporales diferentes. Mientras a la escala geológica, el CO_2 atmosférico supone la principal fuente de carbonatos en las rocas sedimentarias, en una escala temporal de orden menor (decenas a miles de años), el CO_2 atmosférico también contribuye de manera significativa como fuente de C en la vegetación y los suelos del planeta. Este ciclo, conocido como *ciclo corto* del C, es de especial relevancia para el funcionamiento de los ecosistemas terrestres tal y como los conocemos, y puede ser modificado antrópicamente al responder a cambios inducidos por la actividad humana en sus tres reservorios principales (atmósfera, vegetación y suelo). Dentro de este ciclo, el suelo (pedosfera) es un componente de gran importancia ya que es el segundo reservorio en tamaño, y el flujo entre atmósfera y pedosfera es uno de los principales del ciclo (Lal et al., 1997).

En el suelo, el carbono puede encontrarse tanto en forma orgánica (SOC) como inorgánica (SIC). El conocimiento actual sobre el reservorio en forma de SIC es muy reducido, aunque hay estimaciones que lo sitúan alrededor de un 12% mayor que el reservorio de SOC, que está estimado en 1500 Pg (Lal et al., 1997). Esto supone que el reservorio en forma de SOC es 2.1 veces superior que el atmosférico y 2.7 superior al biótico. Cambios en el ciclo del carbono orgánico del suelo, que propicien su incremento o su reducción, tendrán, por lo tanto, implicaciones importantes en la calidad medioambiental global y en la composición gaseosa de la atmósfera. Además, si bien los componentes orgánicos (SOC) del suelo son cuantitativamente minoritarios, juegan un papel esencial en la génesis y estabilización de los suelos, como se explica más

adelante, así como en el desarrollo de la vida en ellos. Por lo tanto, variaciones del stock de SOC pueden tener también importantes efectos en la calidad del suelo relacionados con su estructura, biodiversidad, capacidad de retención de agua y su disponibilidad, profundidad de enraizamiento y ciclo de los nutrientes (Wander et al., 2004).

El SOC se renueva en un ciclo dinámico, de manera que la cantidad (stock) de SOC en un momento dado viene determinada por el contenido inicial, las entradas al suelo, y las pérdidas desde el momento inicial (Paustian et al., 1997; West & Post, 2002). La principal vía de entrada de C orgánico en suelos son los residuos vegetales, tanto en forma de residuos muertos de la parte aérea como procedente de la parte subterránea de la planta, en forma de raíces muertas (Gale et al., 2000; Rasse et al., 2005) y de productos excretados por las raíces (rhizodeposición) (Balesdent & Balabane, 1996; Molina et al., 2001).

Las salidas de C del suelo pueden ocurrir a través de dos mecanismos principales. En primer lugar la mineralización de los residuos vegetales y de los compuestos orgánicos presentes en el suelo, que supone su devolución a la atmósfera en forma de CO_2 (Jackson et al., 2003; Martens et al., 2005), y en segundo lugar la erosión, que puede arrastrar tanto restos orgánicos (de la superficie) como materia orgánica ya incorporada al suelo ligada a las partículas minerales.

La mineralización es el principal proceso de pérdida de materia orgánica en la mayoría de los suelos agrícolas (Singer & Munns, 2006). Recibe ese nombre porque constituye en realidad la transformación de los componentes orgánicos en productos inorgánicos. Es un proceso que ocurre de forma natural y es esencial en el sistema suelo, ya que a través de él los constituyentes de los restos vegetales son descompuestos en sus constituyentes minerales, que pueden ser, de esta manera, absorbidos por las plantas y utilizados por otros organismos. En general es un proceso controlado por los microorganismos del suelo, que utilizan la materia orgánica como fuente de energía para sus procesos metabólicos. La importancia de la erosión como proceso de pérdida de materia orgánica del suelo es muy variable. Su incidencia dependerá de

factores ambientales (capacidad erosiva de viento y agua), de las características del propio suelo (principalmente la estabilidad de su estructura), y las condiciones en las que se encuentre (grado de recubrimiento, pendiente, etc.)

Por último, comprender el funcionamiento del ciclo del carbono en el suelo implica también comprender la dinámica de la materia orgánica, y los procesos que ocurren en el propio suelo. La estabilización de la materia orgánica en el suelo ocurre a través de diversos mecanismos, según se explica más adelante. Puede quedar físicamente inaccesible a la degradación biológica, entrando a formar parte de los agregados del suelo, o químicamente, formando uniones electroquímicas (complejos de adsorción de primer y segundo orden) con las partículas minerales que impidan su degradación (Tisdall et al., 1996; Six et al., 2002a). Además, no todos los compuestos orgánicos son degradados por los microorganismos edáficos con la misma facilidad, e incluso la propia síntesis microbiana puede generar subproductos metabólicos de difícil degradación (recalcitrantes), lo que supone una estabilización bioquímica (Wander, 2004).

En el caso de suelos alterados respecto a su estado de equilibrio natural, como los suelos agrícolas, el manejo del suelo tiene efecto en estos mecanismos de estabilización, que pueden favorecer o no el almacenamiento de SOC en el suelo. Así, en los suelos cultivados, el equilibrio dinámico del ciclo del carbono está alterado en dos sentidos. En primer lugar, porque una parte importante de la biomasa vegetal es exportada del sistema cada año a través de la cosecha (se reducen las entradas), y en segundo lugar, porque la alteración física que supone el laboreo puede implicar una aceleración importante de los procesos de mineralización (aumenta la salida). Estos procesos han sido demostrados ampliamente en diversos estudios (Singh et al., 2007), y están en el origen de la pérdida generalizada de materia orgánica en los suelos cultivados (Singer & Munns, 2006) respecto a su situación anterior al cultivo.

Dinámica de la materia orgánica en el suelo. Como se ha señalado, la fracción orgánica del suelo tiene un papel clave en el funcionamiento de éste, al participar de la mayor parte de procesos

que dan al suelo sus propiedades químicas, físicas y biológicas, incluyendo la formación y estabilización de su estructura. Esto es así porque el ciclo de incorporación de la materia orgánica al suelo está estrechamente vinculado a la formación y estabilización de los agregados. Los agregados son 'agrupaciones' de partículas minerales y orgánicas, que se forman como resultado de la interacción de las partículas minerales entre sí y con la materia orgánica (a la que pueden a su vez proteger de la degradación). La materia orgánica contribuye así a la unión de las partículas minerales (Six et al., 1999 y 2004), participando en su propia estabilización (Puget et al., 2000).

Este ciclo "se activa" cuando nuevos restos orgánicos llegan al suelo. Allí son el objeto de una intensa actividad biológica, que resulta por un lado en una transformación de estos restos vegetales en compuestos orgánicos más difícilmente degradables y en partículas orgánicas de menor tamaño, y por otro lado, en la formación de compuestos de síntesis metabólica que facilitan la unión de las partículas minerales más reactivas (generalmente las de la fracción arcilla), con la materia orgánica. Esta unión a la fracción mineral protege a su vez a los componentes orgánicos de la acción degradadora de los microorganismos, y como resultado, la materia orgánica queda paulatinamente protegida en el seno de los nuevos agregados. Así, con el tiempo, dentro de los agregados iniciales más grandes (macroagregados) se forman agregados más pequeños y más estables (microagregados), alrededor de los núcleos de materia orgánica, de manera que para que el estado físico del suelo se mantenga, es necesaria la entrada continua de restos orgánicos que puedan incorporarse a este ciclo y mantengan la estabilidad de los macroagregados. Este proceso de agregación fue descrito por Tisdall y Oades en 1982, y se conoce como teoría jerárquica de los agregados. Posteriormente ha sido corroborada en numerosos estudios (Golchin et al., 1994, Angers & Chenu et al., 1997, Six et al., 1999 y 2004). El término "jerárquico" se aplica en este caso con un doble sentido: en primer lugar, porque se entiende que los agregados estables del suelo de diferentes tamaños tienen un origen diferente y cumplen funciones diferentes en el suelo, pero están relacionados

entre ellos en su formación, de manera que los más pequeños se originan en el seno de los de mayor tamaño. En segundo lugar, existe también una jerarquía en relación a los agentes que estabilizan estos agregados: mientras los más pequeños son estabilizados principalmente por uniones entre la fracción orgánica y mineral, o por interacciones entre compuestos orgánicos de origen metabólico, los de mayor tamaño lo son por componentes de la fracción orgánica de reciente entrada y de duración más efímera, tales como las hifas de los hongos y las raíces de las plantas.

Por lo tanto, la interacción entre materia orgánica y agregación tiene efectos recíprocos, de modo que la entrada continua de materia orgánica al suelo contribuye no sólo al mantenimiento de niveles adecuados para la nutrición de la biota del suelo y las plantas, sino también al desarrollo y estabilización de la agregación del suelo. De manera recíproca, la agregación, según el mecanismo explicado previamente, tiene un papel de gran importancia en la estabilización de la materia orgánica. En la mayoría de estudios en que se ha realizado un fraccionamiento físico del suelo que permita estudiar la agregación, se observa que cerca del 90% de la materia orgánica del suelo se encuentra formando parte de los agregados de éste (Angers & Chenu et al., 1997).

En este proceso de estabilización en los agregados es importante distinguir entre el tipo y la cantidad de materia orgánica retenida en los distintos niveles de agregación. La materia orgánica que queda protegida físicamente en los microagregados (<250 µm) de manera más estable, puede encontrarse tanto en los llamados microagregados libres (no incluidos en agregados mayores) como en microagregados contenidos en el interior de macroagregados (Six et al., 2002a). Esto es debido a que en ellos los poros son de menor tamaño, y los flujos de agua y gases, así como la propia movilidad de los microorganismos se ve limitado.. Además, las uniones químicas entre partículas se ven facilitadas en estas condiciones (Tisdall et al., 1996). En cambio, la materia orgánica que se encuentra en los macroagregados es más joven (Six et al., 2004) y tiene un ciclo de retorno más corto frente a aquella que se encuentra en los microagregados. Esta diferencia se ha comprobado en diversos estudios de dinámica de la materia orgánica, entre otros medios,

mediante el uso de herramientas isotópicas basadas en el estudio de la abundancia natural de los isótopos del C correspondientes a diferentes tipos de plantas (Angers & Chenu et al., 1997). La relación entre la materia orgánica del suelo y las características de su matriz existe no sólo en términos de cantidad almacenada, sino en relación a los tiempos de retorno y la estabilidad físico-química de las diferentes fracciones de la materia orgánica.

Suelos carbonatados. Influencia de la presencia de carbonatos alcalinotérreos. Los suelos carbonatados, muy abundantes en las regiones áridas y semiáridas mediterráneas, presentan en muchas de estas zonas contenidos bajos de materia orgánica debido a la baja productividad primaria de estos ecosistemas, y a un clima que favorece la mineralización (Zornoza et al., 2007). La actividad biológica en estos suelos también está condicionada por las limitaciones hídricas del suelo y las condiciones extremas de temperatura en algunas zonas de estas regiones. Estos suelos son, por lo tanto, especialmente sensibles a cambios en su calidad como consecuencia de un manejo agrícola que incida en el contenido y la calidad de la materia orgánica que contienen. Concretamente, la respuesta de la fracción orgánica y la agregación a aportes orgánicos externos es en general positiva, aunque varía en función del tipo de suelo (Virto et al., 2006). Entre ellos, los suelos de textura gruesa (arenosos), son especialmente sensibles a la degradación relacionada con este ciclo (Quiroga et al., 1996).

Otro aspecto importante en relación a los suelos ricos en carbonatos alcalinotérreos y el ciclo de la materia orgánica, es el ilustrado por Baldock & Skjemstad (2000) en su revisión sobre el papel de la fracción mineral del suelo en la protección de la materia orgánica frente al ataque microbiano. Estos autores repasan las evidencias mostradas hasta la fecha sobre el papel del calcio en la estabilización del C orgánico en el suelo, a partir de los dos mecanismos sugeridos por Duchafour (Baldock & Skjemstad, 2000) la formación de recubrimientos (*coatings*) de carbonatos sobre los residuos vegetales más o menos frescos (visibles por ejemplo en láminas delgadas), y la precipitación de compuestos orgánicos más humificados inducida por la formación de enlaces Ca-materia

orgánica. Esta protección de la materia orgánica del suelo por el calcio frente a la degradación microbiana, ha sido demostrada en estudios de mineralización (Baldock & Skjemstad, 2000). También se ha observado, en estudios recientes, que la relación entre materia orgánica y agregación en este tipo de suelos es menos evidente que en suelos sin carbonatos (Fernández-Ugalde et al., 2011). Esto es debido a que los carbonatos pueden ayudar a la estabilización de macroagregados de manera independiente a la descomposición de la materia orgánica. Además, estas diferencias en la estructura física del suelo, pueden contribuir a la estabilización de la materia orgánica de manera diferente a los casos en los que la materia orgánica es el principal agente de formación y estabilización de la estructura (Kemmit et al., 2008; Fernández-Ugalde et al., 2014.) Este proceso es aún insuficientemente conocido, pero indica la necesidad de tener en cuenta la fracción mineral y la composición de la estructura del suelo a la hora del estudio de la materia orgánica en estas regiones.

Desde un punto de vista práctico, la presencia de carbonatos en la fracción mineral de los suelos también tiene una influencia importante sobre los métodos de medida de la materia orgánica. Así como en suelos sin presencia de carbono inorgánico el uso de la combustión total es reconocido como el más preciso y puede ser utilizado como referencia (Bisutti et al., 2004), la búsqueda de un método rápido y preciso en suelos con presencia de carbono inorgánico supone todavía un reto (Chaterjee et al., 2009). El método de combustión húmeda (Wakley and Black, 1934) es el más utilizado a nivel mundial, pero no garantiza una oxidación completa de la materia orgánica. Por otro lado, el uso del método de combustión total hace necesaria la eliminación previa de los carbonatos presentes en la muestra mediante el uso de tratamientos ácidos, con posibles efectos sobre la materia orgánica. La identificación de métodos precisos y lo más sencillos posibles de análisis y estudio de los componentes orgánicos de suelos con carbonatos es un reto que permanece abierto.

2. Influencia del manejo agrícola en la materia orgánica del suelo.

El contenido de materia orgánica en suelos cultivados es habitualmente más bajo que en suelos no cultivados de las mismas características. En las últimas décadas, se ha realizado un importante esfuerzo de investigación para determinar el grado de "reversibilidad" de esta situación en diferentes agrosistemas y condiciones edafoclimáticas, así como de las técnicas de manejo que podrían revertirla total o parcialmente, devolviendo a los suelos cultivados niveles de materia orgánica equivalentes o incluso superiores a los suelos no cultivados.

Respecto al manejo agrícola, existen varias opciones que han demostrado ser eficaces a la hora de incrementar los contenidos de carbono orgánico en el suelo (SOC): abonado orgánico, enmiendas de compost, sistemas de pradera, cubiertas vegetales y sistemas de no laboreo (Wander, 2004). Estos sistemas actúan sobre el ciclo anterior aumentando las entradas y/o reduciendo las salidas de C orgánico del suelo. Concretamente, los sistemas de no laboreo han sido muy documentados los últimos años aunque existe cierta variabilidad en los datos obtenidos.

Influencia del sistema de laboreo. Como se ha señalado anteriormente, en los suelos cultivados el laboreo repetido provoca cambios importantes en la estructura, alterando la cantidad y distribución de los agregados. Estos cambios afectan, además, de una manera directa a la interacción entre la agregación y la dinámica de la materia orgánica. En primer lugar, las labores de cultivo producen una ruptura de los macroagregados, interrumpiendo el ciclo natural de formación de estructura. Esta menor presencia de macroagregados estables reduce la formación de microagregados en su interior (Six et al., 2004). La ruptura de macroagregados por el laboreo se debe no solamente a su acción física sobre el suelo, sino a cambios en temperatura y humedad, mayor aireación, exposición de nuevos agregados a la superficie y exposición de la materia orgánica a agentes descomponedores (Dalal & Bridge et al., 1996). En segundo lugar, además de la ruptura de agregados, el laboreo induce una disminución de la actividad biológica en el suelo,

principalmente fúngica. En otras condiciones estos organismos son agentes importantes en la agregación, debido tanto a la producción de sustancias cementantes como al desarrollo de un red de hifas que favorece la formación y estabilidad de los agregados (Six et al., 2002b).

La dinámica de la materia orgánica se ve en consecuencia también afectada por estos cambios en la estructura del suelo. La disminución de la agregación lleva consigo una disminución de la materia orgánica del suelo ya que el 90% de ésta se encuentra retenida en los agregados (Angers & chenu et al., 1997), y se ha demostrado también que los macroagregados estables son los más ricos en carbono orgánico (Six et al., 2002a; Balesdent et al., 2000).

De esta manera se puede decir que el laboreo provoca un cambio en la arquitectura del suelo, que tiene como consecuencia una reducción de su contenido en carbono y nitrógeno fácilmente degradables. Como consecuencia de la alteración de la agregación, la tasa de descomposición de la materia orgánica varía también con el laboreo. Los valores de estudios sobre el efecto de las prácticas de no laboreo muestran por ejemplo un tiempo medio de residencia de la materia orgánica 1,5 veces menor en los suelos labrados de manera continua que en los no labrados (Six et al., 2002b).

El estudio de la reversibilidad de las pérdidas de materia orgánica como consecuencia del manejo agrícola implica por lo tanto, el estudio de su relación con la recuperación de la estructura del suelo. Como se ha documentado, en muchos casos los sistemas que incluyen la supresión del laboreo suponen un incremento en el contenido de materia orgánica, aunque no siempre es así. Según describe Franzluebbers et al. (2004), entre los factores que pueden modificar los efectos que tiene el laboreo sobre la relación entre agregación y dinámica de la materia orgánica, los principales son la textura, el clima y la rotación e intensidad del cultivo. Por un lado, los incrementos de agregación y almacenamiento de SOC observados al suprimir el laboreo son mayores en suelos de textura fina que en aquellos de texturas más gruesas, debido a la capacidad inherente de los minerales de la arcilla para interactuar en la formación de agregados. Por otro lado, aunque la relación con el clima no es tan evidente (Virto et al., 2012), y la información que dan estos datos es

limitada, estudios realizados a lo largo de Estados Unidos muestran mayores incrementos en el área de clima mésico subhúmedo. Además, en ensayos que incluyen determinadas rotaciones de cultivos se observan diferencias frente a parcelas con monocultivo, aunque no siempre los incrementos mayores se producen para el mismo tratamiento. Finalmente, la intensidad de cultivo puede afectar ya que sistemas con mayor producción de biomasa tienen, potencialmente, mayor capacidad de secuestro de C, pero faltan datos significativos al respecto. Dos amplios estudios que ofrecen medias cuantitativas son el realizado por West and Post (2002) a escala mundial, con un valor medio de 8,5 Mg C ha^{-1} almacenados durante 15 años en los suelos con reducción o eliminación del laboreo, y el realizado por Franzluebbers (2005) para el sudeste de los Estados Unidos con un valor medio de 3,3 Mg C ha^{-1} en 10±5 años. En ambos estudios se observa una importante variabilidad en la capacidad de las técnicas de laboreo reducido para aumentar el stock de SOC. Esta variabilidad parece indicar que la capacidad de las prácticas de no laboreo para secuestrar carbono orgánico en el suelo depende de las condiciones del agrosistema específicas de cada lugar (Puget & Lal, 2005). González-Sánchez et al. (2012) publicaron recientemente un estudio sobre las posibilidades de secuestro de carbono en España mediante técnicas de agricultura de conservación. El estudio muestra como en la mayor parte de estudios llevados a cabo en diferentes regiones, la transformación a sistemas de no laboreo supone un incremento promedio del carbono orgánico en los primeros 10-15 centímetros de suelo de 0,85 Mg ha^{-1} $año^{-1}$. Por otro lado, dos estudios publicados recientemente en Francia destacan la importancia de la estratificación del carbono en el perfil del suelo que conlleva un aumento del contenido de materia orgánica en la capa superficial del suelo, pero un incremento nulo en el total del perfil de suelo estudiado (Dimassi et al., 2014; Mary et al., 2014). Una revisión reciente (Virto et al., 2012) resalta la importancia de los residuos vegetales que se incorporan al suelo. En él se analizan 92 pares de parcelas con tratamientos comparativos de no laboreo y laboreo con volteo del suelo. Los resultados muestran como la diferencia en la cantidad de carbono orgánico incorporado al suelo es el factor más determinante a la hora de explicar diferencias en el

almacenamiento de carbono orgánico debidas a la conversión a un sistema de no laboreo.

Finalmente, existen ejemplos de determinados tipos de manejo intensivo de suelos agrícolas y de pastos que han resultado incluso en aumentos del contenido de SOC respecto de esos mismos suelos en condiciones vírgenes (Six et al., 2002a). Analizar la influencia del manejo, y considerar los agrosistemas y su manejo desde una perspectiva holística, y no sólo el compartimento "suelo", es por lo tanto imprescindible, a la hora de estudiar el balance de C en suelos cultivados. La cuantificación de los cambios causados por este tipo de manejos en el ciclo del carbono orgánico requiere un control tanto de las entradas potenciales (cambios en la producción de biomasa vegetal), como del stock total en el perfil completo de suelo, que puede verse modificado por cambios relacionados con sus características intrínsecas (agregación, propiedades físicas, actividad biológica). Por último, recientemente, Lal (2015) resalta la necesidad de combinar el no laboreo con otras técnicas de agricultura de conservación (gestión de los residuos de cosecha, rotación de cultivos y cultivos intermedios) para lograr un secuestro de carbono en suelo efectivo.

Influencia del riego. En las zonas áridas y semiáridas del planeta, el principal limitante para el desarrollo vegetal es la cantidad de agua disponible. En estas zonas la instalación de sistemas de regadío permite un incremento de la producción de los cultivos, posibilita la introducción de cultivos con mayores requerimientos de agua y aumenta la seguridad y regularidad de la cosecha. La influencia más evidente de la puesta en regadío de suelos agrícolas ocurre por lo tanto sobre los cultivos, es decir, sobre la producción de biomasa vegetal en el agroecosistema. Como consecuencia de estos cambios, es esperable que el regadío suponga un incremento de la biomasa potencialmente incorporable al suelo. Esto puede ocasionarse como consecuencia tanto de la introducción de nuevos cultivos con mayor producción potencial de biomasa, así como por el aumento productivo de cultivos ya presentes en el secano. Estudios anteriores del ciclo del carbono orgánico, en los que se ha controlado la biomasa vegetal incorporada al suelo, muestran como el regadío ha supuesto incrementos significativos importantes de la biomasa

vegetal incorporada. En Estados Unidos se contabilizaron los inputs de carbono orgánico en distintas parcelas de Nebraska y Colorado, durante 5 años, en rotaciones de cultivos diferentes. El incremento de biomasa vegetal incorporada en regadío frente a secano fue en valor medio de 314 g C m^{-2} $año^{-1}$ en Nebraska y de 288 g C m^{-2} $año^{-1}$ en Colorado (Denef et al., 2008). En otro estudio llevado a cabo en la misma zona, la diferencia de carbono incorporado en una rotación de regadío y una rotación de secano en la misma parcela fue de 273 g C m^{-2} $año^{-1}$ (Gillabel et al., 2007). Algunos estudios indican sin embargo que la biomasa vegetal de cultivo no aumenta en la misma proporción que la producción de grano, debido a que es habitual un aumento del índice de cosecha (IC) en condiciones de regadío (Giaveno et al., 2002). Este índice relaciona la producción de grano con la biomasa total producida por el cultivo.

El aumento en la cantidad de biomasa producida y potencialmente incorporable al suelo no es, sin embargo, el único impacto sobre el suelo de la puesta en regadío de terrenos agrícolas. Las consecuencias de este cambio pueden suponer tanto una mejora en la calidad de los suelos, como ser causa de procesos que lo degraden. Esto es así porque los efectos resultantes de esta transformación son función de diversos parámetros como el tipo de suelo y su manejo, el tipo de fertilización y el tipo de rotación. Estos efectos son muy variados y afectan no sólo a la fracción orgánica, como se ha expuesto, sino también a diferentes características y procesos del suelo (propiedades químicas, propiedades físicas, agregación, actividad biológica, etc.). Además, estas características y procesos edafológicos se encuentran relacionados entre sí. El conocimiento existente sobre las consecuencias del cambio secano-regadío sobre la fracción orgánica es todavía sin embargo insuficiente. Por todo ello, es complejo predecir el efecto neto que puede tener la puesta en regadío sobre un suelo agrícola, ya que es muy posible que éstas varíen según las condiciones de cada lugar. Será necesario, por lo tanto, realizar estudios locales y a nivel de campo para evaluar con precisión su efecto en las diferentes regiones del planeta. En general, los dos aspectos más relevantes en relación a esta transformación son los posibles cambios físico-químicos asociados a problemas de salinización y sodificación, los

relacionados con la física del suelo (degradación de la estructura, encostramiento, aumento de la erosionabilidad, etc), y los producidos en el ciclo de la materia orgánica.

En zonas áridas y semiáridas, el riego con aguas de calidad baja puede provocar un aumento del sodio intercambiable en el complejo de cambio, lo cual acelera los procesos de degradación física del suelo antes mencionados. Esto es debido principalmente al incremento de la dispersión de las partículas de arcilla y de los complejos órgano-minerales, y a la reducción de la resistencia de los macroagregados al impacto de las gotas de lluvia y riego (Amezketa, 1999). Los procesos de salinización también pueden ocurrir por ascenso capilar, cuando los sistemas de drenaje no están bien diseñados, o las condiciones de riego no contemplan las dosis y volúmenes adecuados.

Los procesos relacionados con la estructura y las propiedades físicas del suelo pueden verse fuertemente afectados con la puesta en regadío de terrenos agrícolas. Diversos estudios han demostrado importantes cambios en propiedades físicas, tales como aumentos de la densidad aparente, y reducción de la porosidad y la conductividad hidráulica (Gillabel et al., 2007). También se han documentado procesos de encostramiento y sellado superficial debidos al impacto de las gotas de agua (Gillabel et al., 2007). Por otro lado, la puesta en regadío podría tener importantes efectos sobre el ciclo de la agregación, aunque por el momento han sido objeto de pocos estudios. Estos efectos están principalmente ligados a cambios en el ciclo de la materia orgánica, ocasionados por el incremento de las entradas de residuos orgánicos (Follet et al., 2005; Adviento-Borbe et al., 2007), y el incremento de la actividad microbiana (Gillabel et al., 2007; Denef et al., 2008). Estos cambios pueden generar un aumento del contenido de materia orgánica en el suelo (Gillabel et al., 2007; Denef et al., 2008), y potenciar el ciclo de la agregación. Otro proceso alterado por el regadío, y que afecta directamente a la estructura, son los ciclos de humectación-secado del suelo ya que la implantación del regadío aumenta su frecuencia pero disminuye su intensidad. Se ha observado que esta disminución de la intensidad de estos ciclos puede favorecer el proceso de agregación (Gillabel et al., 2007), debido a que se ralentiza el periodo de retorno de los

macroagregados, favoreciendo así la formación de microagregados en su interior. Este proceso seguramente depende de diversos factores del suelo (Six et al., 2004), y parece tener efecto únicamente en el corto plazo (Denef et al., 2001). Por último, hay autores que resaltan el papel que juegan los mecanismos de erosión y sedimentación, la erosión hídrica en suelos de regadío, por ejemplo, puede ser un mecanismo de pérdida de carbono orgánico, aunque en parcelas niveladas es poco importante (Poch et al., 2006),

La materia orgánica aparece por lo tanto como uno de los componentes del suelo con mayor respuesta a los cambios de manejo, aunque es difícil predecir el sentido y magnitud de estos cambios. El efecto de la puesta en regadío ha sido, hasta ahora, poco documentado. Sin embargo la bibliografía existente, que coincide en que el regadío provoca un incremento en la producción de biomasa y un aumento potencial en las entradas de materia orgánica al suelo, también documenta en general un incremento en las tasas de mineralización de la misma (Watson et al., 2000).

En este sentido, y considerando que muchas zonas que se transforman al regadío tienen suelos generalmente pobres en materia orgánica, un aumento de las entradas de biomasa del cultivo puede generar un incremento en el contenido de materia orgánica en el suelo (Gillabel et al., 2007). Sin embargo, este incremento está condicionado por el tipo de cultivo y el sistema de producción, fertilización y laboreo (Follet et al., 2005), hasta el punto de que en ocasiones, el tipo de manejo y la rotación de cultivos utilizada puede suponer un aumento nulo o la disminución del carbono orgánico en el suelo (Adviento-Borbe et al., 2007). En consecuencia, el regadío puede suponer un incremento en el almacenamiento de carbono orgánico en el suelo, siempre y cuando vaya acompañado de un sistema de producción que propicie un aumento de la biomasa incorporada al suelo y su estabilización. En situaciones en las que el sistema de producción en regadío suponga una degradación física o química de las propiedades del suelo, como las observadas por Nunes et al. (2007) en el sudoeste de la península Ibérica, el regadío puede provocar una disminución del contenido de materia orgánica del suelo.

Por otro lado, el aumento de la tasa de mineralización de la materia orgánica como consecuencia de la puesta en regadío, ha sido medido en algunos estudios a través de la tasa de emisión de CO_2 (Jackson et al., 2003; Martens et al., 2005). Este aumento se debe a un incremento de la actividad microbiana responsable de la mineralización de compuestos orgánicos que se ve favorecida tanto por el aumento y continuidad de la humedad del suelo, como por el aumento de los restos vegetales disponibles. Este aumento de la mineralización puede contrarrestar el efecto del aumento de la biomasa vegetal incorporada al suelo, mitigando el almacenamiento de materia orgánica en el suelo en algunos casos (Denef et al., 2008). La actividad microbiana también puede tener sin embargo otros efectos que resultan positivos en relación al aumento del contenido de materia orgánica en el suelo. Gillabel et al. (2007) se plantearon como hipótesis que el aumento de la actividad microbiana junto con el aumento de biomasa vegetal incorporada pueden generar un cambio en la dinámica de agregación y estabilización de la materia orgánica en los agregados, lo que podría resultar en una mayor estabilización de carbono orgánico en los microagregados.

Caso de estudio. Calidad del suelo, dinámica de la materia orgánica y secuestro de carbono en los regadíos de Navarra[1].

La puesta en marcha de la infraestructura de riego "Canal de Navarra" ha supuesto un aumento importante de la superficie agrícola en regadío en la región (más de 53.000 hectáreas). Además del importante efecto socioeconómico sobre el sector agrario, está transformación conlleva un notable impacto medioambiental que debe ser analizado. En especial el efecto sobre el suelo, un bien natural esencial para la actividad agraria. Considerando lo anteriormente expuesto sobre la necesidad de evaluar a un nivel local los efectos potenciales de la transformación en regadío de los suelos agrícolas de secano, en 2009 se inició un proyecto conjunto entre INTIA (Instituto Navarro de Tecnologías e Infraestructuras Agroalimentarias)

[1] Este epígrafe recoge, de manera resumida, la información publicada en la siguiente referencia: Apesteguía, M.; Orcaray, L.; Virto, I.; Bescansa, P.; Enrique, A. 2014. Impacto del regadío en suelos de secano. Navarra Agraria 206:32-36.

y el grupo de manejos sostenible de suelos de la UPNA (Universidad Pública de Navarra), financiado por INIA (Instituto Nacional de Investigaciones y Tecnología Agraria y Alimentaria). El proyecto puso el foco en el efecto del regadío sobre la calidad del suelo, la dinámica de la materia orgánica en el suelo y su capacidad para secuestrar carbono atmosférico. A la luz del conocimiento expuesto anteriormente, se estableció la hipótesis general de que en las condiciones agroclimáticas de las nuevas zonas regables de Navarra, con suelos pobres en materia orgánica, de pH básico y ricos en carbonatos, la implantación del regadío puede favorecer la estabilización de la materia orgánica, aumentando la calidad del suelo y resultando en un secuestro efectivo de C atmosférico. Considerando que en investigaciones realizadas anteriormente por los equipos de investigación de este proyecto, se había observado a nivel de parcelas de cultivo una respuesta diferente en función de las unidades de suelo, su manejo y el tipo de cultivo, se vió la necesidad de establecer ensayos de campo *ad hoc*, a fin de contrastar la evolución observada de la dinámica de la materia orgánica tras la implantación del regadío y su interacción con el manejo agrícola. Estos ensayos deben servir para contrastar los datos recogidos en parcelas de cultivo controladas, a fin de obtener una visión completa del efecto del regadío en la calidad de los suelos de la región, en el secuestro efectivo de C, y de los factores que lo condicionan.

El **objetivo principal** del Proyecto fue la **evaluación del impacto del regadío en la calidad de suelos semiáridos del centro y sur de Navarra, a través del estudio de la dinámica de la materia orgánica y de otros indicadores de calidad del suelo,** así como del análisis del contenido de carbono estabilizado en los suelos de regadío.

Para ello, en el proyecto se estructuró en tres líneas de trabajo. La línea 1 permitió recabar datos de distintas zonas de regadío en Navarra y obtener información a nivel regional, mientras que las líneas 2 y 3 basadas en ensayos experimentales en campo permitieron profundizar con más detalle en el ciclo del carbono en la agricultura de regadío. El trabajo científico realizado en la línea 3 se detalla en el capítulo 2 de esta tesis, por lo que se exponen a continuación los resultados principales de las líneas 1 y 2, como

ejemplo de un caso de estudio sobre las consecuencias de la transformación en regadío de agrosistemas de secano.

Línea 1. Estudio de parcelas en nuevos regadíos del Plan de Regadíos de la Comunidad Foral de Navarra.

Las parcelas de cultivo seleccionadas se localizaron en **tres zonas de Navarra**, en regadíos de diferente antigüedad y características edafoclimáticas. Se trata de los regadíos **de Funes Alto (Las Suertes), Santacara y Valdizarbe.** La Figura 1 recoge la localización y características climáticas de estas zonas, así como la situación de las parcelas. Puede observarse que **las tres zonas difieren en aridez** (que aumenta de Valdizarbe a Funes) **y en la duración del tiempo en regadío** (que aumenta en el mismo sentido).

Los suelos en las tres zonas presentan algunas características comunes (elevado contenido en carbonatos y textura franca o franco-arcillosa), con bajos contenidos de materia orgánica en el horizonte superficial.

El trabajo realizado sobre estas parcelas consistió en una **monitorización anual del contenido de materia orgánica (C orgánico) y de su fracción más fácilmente biodegradable** ("C en la materia orgánica particulada", que es muy sensible a los cambios en el manejo del suelo), y en un registro del cultivo implantado cada año. Previamente, en cada una de las parcelas se realizó una **caracterización completa del suelo**, con la idea de poder establecer los factores que más influyen (de suelo, de clima o de cultivo) en el almacenamiento de C orgánico en estos suelos de regadío.

De manera complementaria se realizó un **estudio de la calidad física del suelo a través de la evolución de su estructura.** Asimismo, en la zona de **Funes Alto**, además de las parcelas de regadío se estudió una parcela adyacente que permanece en **secano,** y que previamente a la transformación conformaba una única unidad de trabajo con una de las parcelas de regadío.

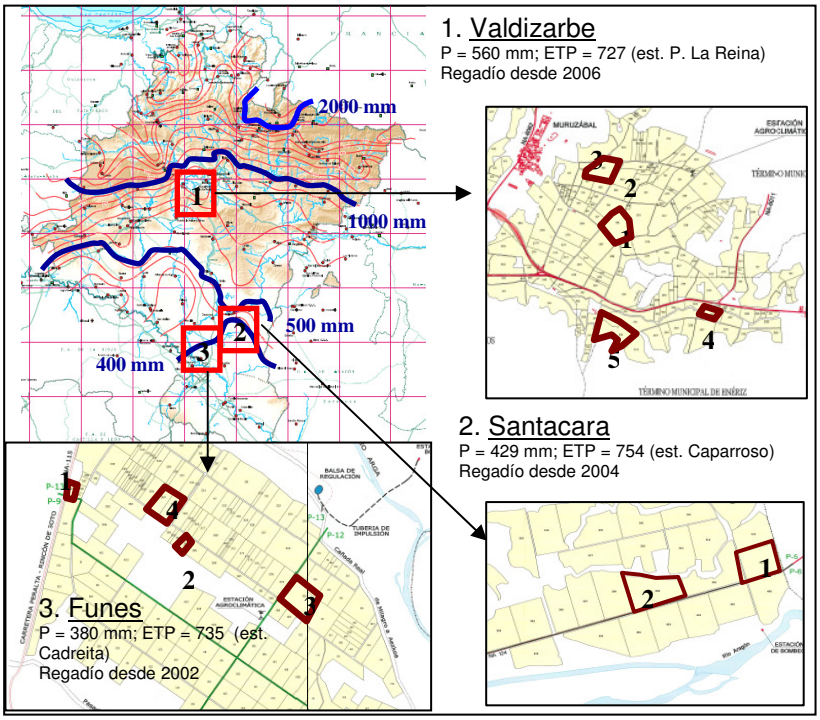

Figura 1. Localización y características de las tres zonas de estudio, y de las parcelas de control dentro de ellas.
P = precipitación media anual. ETP = evapotranspiración media anual.

Resultados principales Línea 1

En primer lugar, hay que destacar que el contenido de materia orgánica en las tres zonas estudiadas presentó **contenidos de C orgánico equivalentes a zonas más húmedas**, lo que sugiere que una consecuencia del regadío en esta zona es el aumento de la cantidad de C orgánico almacenada en el suelo. La **comparación** realizada **entre secano y regadío en Funes Alto** (zona más árida), mostró que la incorporación de C orgánico en el regadío fue **mayor durante los primeros años.** Las tasas de esta incorporación no pueden determinarse con precisión, por carecer de la información de la situación de partida de las parcelas (secano). La estimación realizada a partir de la parcela contigua en secano arrojó un secuestro de 19,4 Mg C/ha durante 7 años en los primeros 20 cm del suelo (horizonte labrado, Figura 2).

En cuanto a los tres factores estudiados en las parcelas de regadío incluidas en esta línea (suelo, clima y cultivos), **las propiedades del suelo fueron el principal factor condicionante**

del almacenamiento de materia orgánica tras la puesta en regadío. Esto se confirmó a partir de varias observaciones: escasa variación en el tiempo de los contenidos en las parcelas a pesar de los cambios anuales de manejo, correlación de la concentración de C orgánico únicamente con una propiedad edáfica (contenido en arcilla), y diferencias notables en el contenido de C en dos zonas diferentes, correspondientes a dos tipos de suelo, en una misma parcela (con igual manejo y clima).

Por otra parte, el estudio comparativo de la estructura del suelo en secano y regadío en Funes Alto, **mostró que el regadío generó una estructura más estable (Figura 3).** Esto tiene varias consecuencias. Por un lado, como se ha comentado, fomenta la estabilización de la materia orgánica en el suelo. Además, le otorga una mayor resistencia a la erosión y reduce la formación de costras superficiales.

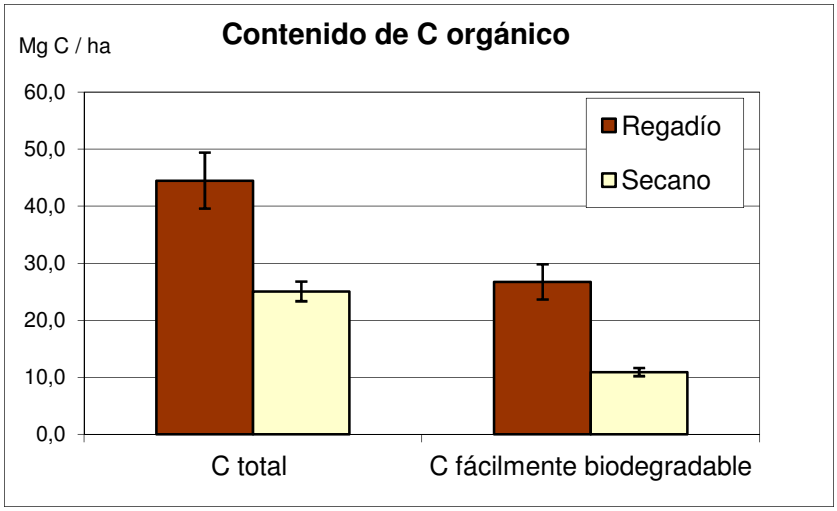

Figura 2. Contenido de C orgánico y de C fácilmente biodegradable en una masa equivalente a 0-20 cm en Funes regadío y secano.

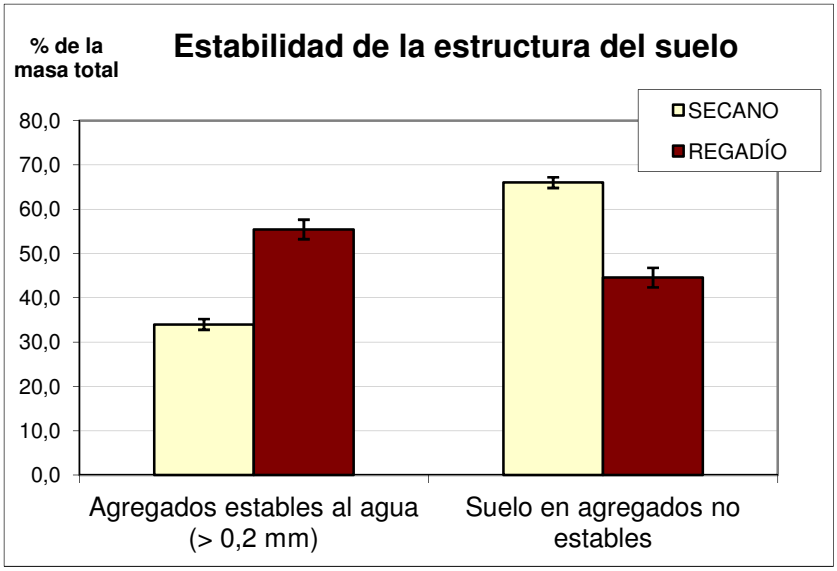

Figura 3. Estabilidad de la estructura del suelo en Funes regadío y secano.

Línea 2. Estudio de la evolución de las propiedades del suelo durante su transformación de secano a regadío en una parcela experimental de larga duración. Interacción del manejo del suelo y el riego.

La línea 2 se desarrolló en una **parcela experimental localizada en el término de Olite (Figura 4).** Se trata de una parcela en la que desde el año 1994 se han ensayado técnicas de Agricultura de Conservación en secano. Coincidiendo con el inicio del Proyecto, esta parcela se transformó al regadío, por lo que se monitorizaron las propiedades del suelo tras esta transformación.

El diseño experimental de esta parcela es en bloques al azar (n = 3), y los tratamientos considerados fueron **No laboreo (NL), Laboreo convencional con vertedera (LC)**, **Laboreo mínimo con chísel (LM) y No Laboreo con quema del rastrojo (NLQR).** El cultivo fue trigo todos los años de estudio. Se realizó un **control anual de las fracciones de la materia orgánica**, y una valoración final de otros indicadores relacionados. En concreto, se estudió de nuevo la estructura (**agregación) y su relación con la materia orgánica**, tanto en la situación de secano inicial como tras dos años de regadío.

Figura 4. Detalle parcela experimental en la ortofoto color del año 2010. Escala 1:1000 (Fuente: SITNA, 2012).

Resultados principales. La introducción del regadío tuvo efectos diferentes sobre la materia orgánica en los diferentes tipos de laboreo. Los sistemas sin laboreo (NL y NLQR) fueron los únicos con un aumento significativo de su contenido de C orgánico tras su puesta en riego (Figura 5), lo que puede asociarse a su mayor capacidad para estabilizar la materia orgánica incorporada. Además, la introducción del regadío supuso una incorporación más homogénea y estable de la materia orgánica en el suelo.

Por otra parte, la agregación y estructura del suelo mejoraron significativamente bajo NL y NLQR, y empeoraron en el manejo convencional (LC), a partir de la situación inicial en secano.

En resumen, estos resultados permitieron observar que la transformación a regadío puede incrementar el contenido de materia orgánica en el suelo, principalmente en zonas áridas donde resulta determinante para un aumento de la productividad primaria del ecosistema. Además, estos cambios en la fracción orgánica del suelo han demostrado tener efectos positivos sobre su estructura, ya que han propiciado un aumento en la agregación que a su vez contribuye en la estabilización a largo plazo de la propia materia orgánica. En cuanto a los factores que condicionan la estabilización de materia orgánica en regadío, las características intrínsecas del suelo (contenido en arcilla) han resultado determinantes. Por último entre los factores ligados al manejo agrícola, han destacado las técnicas de

laboreo de conservación como favorecedora de la estabilización de la materia orgánica tras la transformación a regadío.

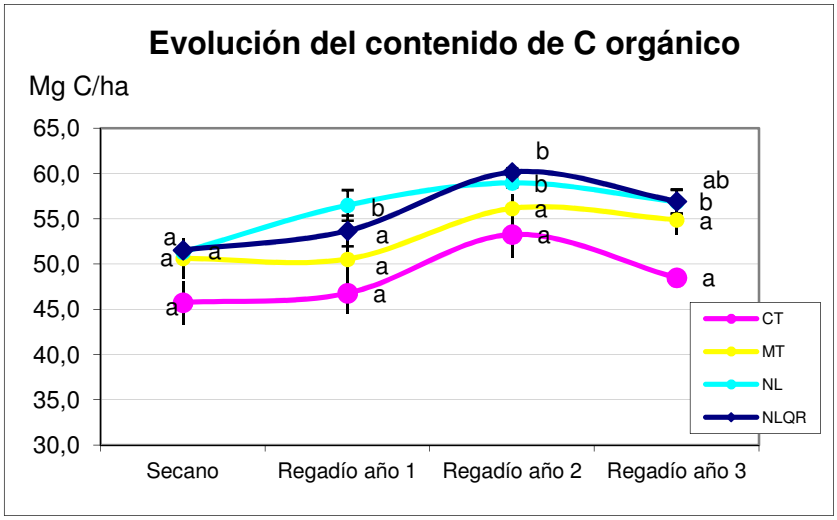

Figura 5. Evolución del contenido de C orgánico en una profundidad equivalente a 0-30. CT: laboreo convencional; MT: laboreo mínimo; NL: no laboreo; NLQR: no laboreo con quema de rastrojo.
Letras distintas en una misma curva representan diferencias significativas en el tiempo (P<0,05).

3. La calidad del suelo

Durante décadas, las prácticas agrícolas se enfocaron principalmente hacia la productividad de los cultivos, con el objetivo de asegurar la obtención de alimentos y otras materias primas necesarias. Sin embargo, el efecto negativo de algunas de estas prácticas sobre bienes naturales esenciales, en especial el agua y suelo, propiciaron, en algunos casos muy poco tiempo después de la implantación de sistemas intensivos, la búsqueda de prácticas agrícolas sostenibles que no comprometan la calidad medioambiental y la productividad a largo plazo. En este contexto, se desarrolló a finales del siglo XX el concepto de calidad de suelo, así como la búsqueda de una metodología apropiada para su estudio. A pesar de que el estudio de suelos según su aptitud agronómica se había realizado durante todo el siglo XX, no fue hasta los años '90 cuando se puso el foco en el

estudio de la calidad del suelo de una manera más integral. En 1997 se produjo un avance importante en el desarrollo del concepto de calidad de suelo cuando Karlen et al. (1997) la definieron de manera extendida y englobando sus aspectos más importantes: productividad, calidad medioambiental y bienestar humano. Así, la calidad del suelo quedó definida como *la capacidad de un suelo específico para funcionar dentro de los límites de un ecosistema natural o controlado, que preserva la productividad vegetal y animal, conserva o mejora la calidad del agua y el aire, y promueve el bienestar humano.* Varios conceptos importantes pueden extraerse de esta definición: 1) la calidad del suelo debe ser evaluada basándonos en su función o funciones, en su capacidad para funcionar (Doran et al., 1996), 2) debe ser evaluada para un suelo específico, ya que depende de sus capacidades inherentes y no pueden generalizarse estándares universales de calidad y 3) depende de las características del ecosistema en que se encuentra y por lo tanto puede verse modificada si estas características varían. El reconocimiento de la multifuncionalidad del suelo es otro avance importante, ya que como resaltaron Singer y Erwin (2000), el enfoque basado en la productividad como única función del suelo había conllevado graves problemas de degradación del mismo. Por ello, y basándose en las ideas de Doran y Parkin (1994), la comunidad científica hizo un esfuerzo en sintetizar las funciones principales del suelo, que engloban como las más significativas, las siguientes: (1) Sustentar la productividad, diversidad y actividad biológica, (2) Mantener la calidad del agua y el aire, (3) Favorecer el reciclaje y filtrado de diversos residuos orgánicos e inorgánicos, (4) Participar en el ciclo de diversos nutrientes y elementos, y (5) Facilitar soporte de infraestructuras y viviendas.

Por último, es importante señalar que el estudio de la calidad del suelo a partir de este marco conceptual resulta en considerar dos componentes dentro de la calidad de un suelo: por un lado la calidad inherente, y por otro lado la calidad dinámica (USDA Soil Quality Institute, 2008). La primera depende de las características intrínsecas del suelo (material parental, topografía, clima). Por lo tanto, es difícil observar cambios significativos en el tiempo, salvo bajo circunstancias de intervenciones humanas o naturales muy disruptivas. En cambio, la calidad dinámica correspondería a aquella que puede modificarse dependiendo del uso y manejo del suelo.

Estudio de la calidad del suelo. Debido a la complejidad y variabilidad de los suelos y a la diversidad de funciones que desempeñan, no hay un método universal estandarizado para evaluar su calidad. Sin embargo, cada vez existe un mayor consenso en la comunidad científica acerca de los criterios básicos que deben cumplirse para su estudio. En concreto, para evaluar la calidad de suelo es necesario seleccionar una serie de variables del suelo que sirvan como indicadores de su calidad (Bautista et al., 2004). Además estos indicadores deben seleccionarse en cada caso, de tal manera que representen las condiciones locales y las características particulares del suelo (Govaerts et al., 2006; Rezaei et al., 2006; Shukla et al., 2006), así como la función que se le otorga (Andrews et al., 2004). No obstante, como ya expresaron Doran y Parkin (1994), las propiedades del suelo para ser consideradas indicadores de calidad deben cumplir una serie de condiciones: deben integrar propiedades físicas, químicas y biológicas del suelo que describan los procesos que tienen lugar en el ecosistema, deben ser sensibles tanto a las variaciones debidas al clima o al manejo, como a los procesos de degradación del suelo de origen antrópico. Por último los indicadores deben ser fáciles de entender, ser accesibles a la mayor cantidad de usuarios posible y cuando sea posible, ser componentes de alguna base de datos de suelo ya existente.

La evaluación de la calidad del suelo según la metodología planteada por Karlen et al. (2003) y ampliada posteriormente por Andrews et al. (2004) consta de varias fases. Una vez se han seleccionado los indicadores más apropiados, éstos conforman un conjunto mínimo de datos, y cada indicador debe ser cuantificado, y en una segunda fase sus valores son normalizados. La normalización de los valores permite integrarlos en un índice, obteniendo así un índice global de la calidad del suelo, que por último deberá ser interpretado para determinar a qué nivel está funcionando ese suelo. En los últimos años se ha avanzado en el desarrollo de este tipo de índices de calidad que integran parámetros físicos, químicos y biológicos del suelo. En la forma de herramientas de asesoramiento para el manejos de suelos varios índices has sido propuestos. Como ejemplo, el *Soil Management Assesment Framework* (SMAF,

Andrews et al., 2004) se ha extendido a lo largo de Estados Unidos y ha sido probado en diversas localizaciones. No obstante, la validez del uso de estos índices necesita ser comprobado para las condiciones locales e interpretado por expertos.

OBJETIVOS de la TESIS

El objetivo general de esta tesis es evaluar la dinámica de estabilización de la materia orgánica en suelos agrícolas calcáreos de clima mediterráneo semiárido, y estudiar el papel que desempeña en la calidad global del suelo y el ciclo del carbono. Con este fin, se plantean los siguientes objetivos específicos que se desarrollan en los diferentes capítulos:

1. Identificar y cuantificar los cambios inducidos por el riego en la dinámica de agregación y de la materia orgánica mediante el uso de herramientas de análisis isotópico en un suelo carbonatado mediterráneo.

2. Determinar las consecuencias de la diferente fábrica y estructura física de los suelos con carbonatos, respecto a los que no los contienen, sobre la disponibilidad biológica de la materia orgánica.

3. Evaluar la capacidad de técnicas de análisis térmico combinadas para la cuantificación simultánea de carbono orgánico e inorgánico en muestras de suelos carbonatados.

4. Estudiar la calidad global de un suelo mediterráneo cultivado bajo distintos manejos del laboreo mediante un índice de calidad de suelo reconocido internacionalmente (SMAF), y evaluar su adecuación a nuestras condiciones agroclimáticas.

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Chapter II

Effect of the conversion to irrigation of semiarid Mediterranean dryland agrosecoystems on soil carbon dynamics and soil aggregation

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ABSTRACT

Irrigation is known to influence soil organic matter dynamics and aggregation, but its effects are not completely understood. In this work, organic C inputs from crops and the topsoil (0-10 cm) organic fraction and aggregates size-distribution were measured during the two first growing seasons in an experimental field with four treatments: rainfed and irrigated wheat (*Triticum aestivum* L.), and rainfed and irrigated maize (*Zea mays* L.). Crops inputs were calculated from grain yields and harvest index data. The organic C stock was measured for an equivalent soil mass from C concentrations and bulk density. Aggregates size-fractions were obtained with a method based in the hierarchical model of aggregation. Maize-derived C incorporated was quantified using $\delta^{13}C$ concentrations in soil samples, because maize residues have higher $\delta^{13}C$ values than C3 plants. Despite differences in yield, no differences were observed in total C inputs to the soil from crops and in organic C stocks or aggregate-size distribution. However, the proportion of maize-derived organic C was higher in irrigated than in rainfed plots (13.4 % and 4.94 %, respectively), and maize-C was observed to incorporate preferentially into large macroaggregates (2000-5000 μm). Incorporation of maize-C in small macroaggregates and microaggregates (250-2000 μm and 50-250 μm) was observed only under irrigation, supporting the hypothesis that irrigation can change the incorporation and mineralization dynamics of crop residues into the soil organic pool and modify their distribution within the soil structure.

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INTRODUCTION

In arid and semiarid regions, agriculture is mostly limited by the availability of water. Irrigation is frequently implemented to increase crop yields and the variety of possible crops. The extent of irrigation has doubled in the last 50 years, leading the total irrigated agricultural surface to over 300 million hectares worldwide (FAO, 2011). Apart from increasing crop yields, the conversion from dryland to irrigation can have other consequences in the agroecosystems. From the point of view of soil functioning, changes in soil organic matter (SOM) dynamics, as a result of the new water and crop management regimes can be expected, when dryland soils are converted to irrigation. Recent studies have shown that irrigation can also affect the soil microbial pool (Holland et al., 2013; Zhang et al., 2014). Figure 1 summarizes the basic changes expected upon irrigation adoption in dryland agroecosystems. The increment of organic inputs to the soil from crop residues (Follet et al., 2005; Adviento-Borbe et al., 2007), and the increase of the soil microbial activity due to increased crop residues availability and less restrictive moisture and nutrients conditions (Gillabel et al., 2007; Denef et al., 2008; Cotton et al., 2013), are the two major changes that can affect organic matter dynamics (Figure 1). Since in most soils organic matter decomposition and aggregation are linked (Golchin et al., 1994, Six et al., 2004), changes in the soil physical condition can also be expected when irrigation is implemented (Figure 1). In particular, it is known that changes in soil management resulting in new organic matter incorporation regimes can induce differences in soil aggregation (e.g. Six et al., 2000).

Although few studies have been conducted so far to investigate the extent of the potential impacts of irrigation in extensive croplands on dryland soils (e.g. Amos et al., 2005; Gillabel et al., 2007; Denef et al., 2008), most authors agree that these effects depend on the management and soil parameters of the respective soil. Fertilization and soil management associated to irrigation can therefore modulate the response of the soil to increased crop residues inputs under irrigation, as illustrated in Figure 1. For example, Follett et al. (2005) found that the suppression of tillage and increased N fertilization

resulted in more effective SOC storage in wheat-maize rotations, but had no effect in a wheat-bean rotation in an irrigated Vertisol in Mexico. Follett et al. (2013) found that conventional management induced greater losses of old SOM in irrigated maize than no tillage. Adviento-Borbe et al. (2007) observed that differences in crop C inputs increased SOM in two continuous maize systems, but decreased SOM stocks in a maize-soybean system six years after the introduction of changes in the intensity of management in irrigated agroecosystems in Nebraska. As a result of this variability, the soil organic C (SOC) content is not always increased in the same proportion as crop yields under irrigation (Denef et al., 2008). In relation to aggregation, increased microbial activity along with an increase in the crop biomass inputs can lead to a change in the aggregation dynamics in irrigated soils (e.g. Gillabel et al., 2007), resulting in a higher stabilization of SOC within the smallest soil aggregates (microaggregates, <250 µm). Increased tillage intensity and/or frequency could, on the other hand, result in significant aggregates disruption (Figure 1). All in all, the impacts of irrigation on organic C dynamics in soils will thus depend on the local field conditions and the characteristics of respective dryland soils. Site-specific research is therefore needed for precisely elucidate this effect.

The main objective of this study was to identify and quantify the changes in organic matter balance, soil organic C dynamics and aggregation induced by irrigation adoption in the short-term (2 years), in a calcareous Mediterranean soil in NE Spain, which had supported dryland crops for decades. An experimental plot in which irrigated and rainfed crops are grown was set with irrigated and rainfed maize (C4 plant) and wheat (C3 plant). Following Figure 1, the hypothesis of the study is that changes in the agroecosystem associated to the adoption of irrigated agriculture may result on changes in organic C dynamics and storage, and in aggregation. In particular, the hypotheses were i) greater organic C inputs and organic C stocks under irrigation, in the plots where the crops primary productivity (yield and biomass) is increased with irrigation, ii) a faster pace of organic matter incorporation into the soil in irrigated maize agroecosystems

because of improved conditions for microbial activity and greater inputs from crops, and iii) enhanced incorporation of organic C from maize into smaller soil aggregates in the irrigated treatment.

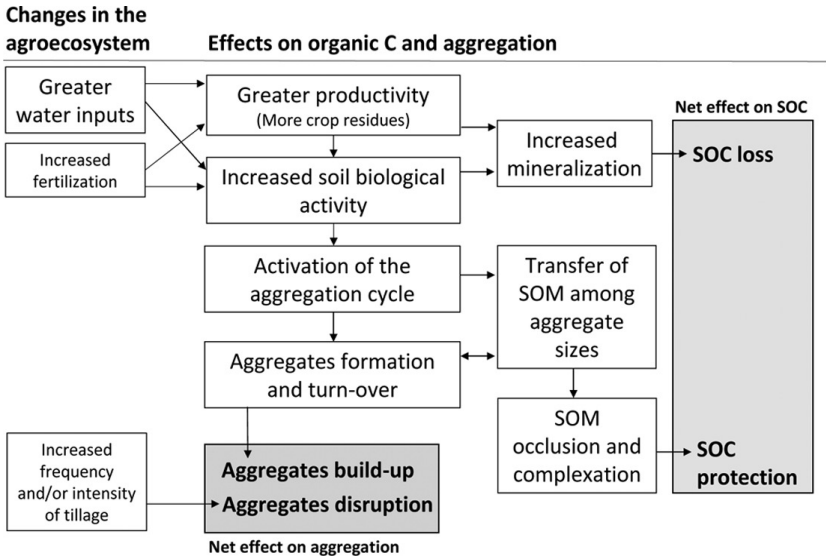

Figure 1. Schematic representation of major changes expected upon irrigation adoption in dryland agroecosystems, and their possible effects on the soil organic fraction (SOM), organic C stocks (SOC), and aggregation.

MATERIALS AND METHODS

Site, treatments and soil sampling. The experimental field was located within an area recently converted to irrigation in Navarre, NE Spain (42° 40' 18,74"N; 1° 45' 4,65"W). Climate is dry temperate Mediterranean (mean annual temperature of 13.4 °C and precipitation of 560 mm). Annual potential evapotranspiration is 718 mm. The soil is a *Calcic Haploxerept* (Soil Survey Staff, 2006), developed on carbonate-rich siltstones and sandstones. It is a fine-loamy deep soil, with 37 % carbonates (pH = 8.2), average clay content of 29 %, and average SOC content of 1.1 % in the 0-10 cm depth. This field has been managed for at least five decades as a traditional cereal dryland field, conventionally tilled (chisel plow) and cultivated with a rotation of winter wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and sunflower (*Helianthus annuus* L.). The experiment was specially designed to evaluate the response of the soil to the adoption of

irrigated agriculture, in a randomized split plot design with irrigation and crop as main factors and three replicates (n=3). Plots were 10 x 6 m. Crops were winter wheat (*Triticum aestivum* L.) and maize (*Zea mays* L.), a C3 and C4 plant, respectively, corresponding to the two most widespread extensive crops in the area. Treatments were: rainfed wheat (W-rf), irrigated wheat (W-irr), rainfed maize (M-rf) and irrigated maize (M-irr). Sprinkler irrigation was used in this field. Soil and crops management were conducted following the local conventional practices (Table 1). Irrigation in W-irr was used in March, April and May. In the two years of the study, monthly precipitation plus irrigation (in brackets) rates were 41 (28), 80 (40) and 31 (56) L m^{-2} in March, April and May in year 1, 51 (0), 23 (76) and 64 (75) L m^{-2} in year 2. In total, W-irr received 276 L m^{-2} of water in year 1 and 289 L m^{-2} in year 2 in these months. In M-irr Irrigation was continuous from June to September. In year 1, precipitation plus irrigation accounted for 57 (30), 31 (153), 4 (195) and 15 (43) L m^{-2} in June, July, August and September, respectively. In year 2, M-irr plots received 21 (84), 7 (208), 5 (243) and 8 (90) L m^{-2} in the same months.

Table 1. Characteristics of the agroecosystems in the experimental field

	Wheat (W-irr and W-rf)	**Maize (M-irr and M-rf)**
Growing season	November - June	May - November
Tillage	Chisel plow (0-10 cm) after harvest Seedbed in early fall	Moldboard plow (0-25 cm) after harvest Seedbed in April
Sowing	450 seeds m^{-2}	8 seeds m^{-2}
Fertilization		
Nitrogen	60 + 120 kg N ha^{-1} (March and April)	40 + 253 kg N ha^{-1} (May and June)
Phosphorous	45 kg P_2O_5 ha^{-1} (November)	101 kg P_2O_5 ha^{-1} (May before sowing)
Potassium	not added	132 kg K_2O ha^{-1} (May before sowing)
Weed control	Conventional herbicides	Conventional herbicides

Undisturbed (100 cm^3 soil cores for bulk density) and disturbed composite samples were collected in each plot at 0-10 cm before the start of the experiment (year 0, baseline), and two cropping seasons after the beginning of the study (year 2). In year 2, sampling

was done after the incorporation of the crop biomass to the soil through tillage, and when the soil moisture conditions granted sampling with the minimum possible soil structure disturbance. Due to the different growing seasons in wheat and maize (Table 1), soil sampling was carried out in fall (November 9) for wheat plots, and in spring (March 28) for maize plots. To ensure equal conditions, thermal integrals were calculated for each crop, so that the number of degree-days accumulated between residue incorporation and sampling was equivalent for the two crops (data not shown). For disturbed samples, five sub-samples were collected with a shovel at random per plot and gently forced to pass through a 5 mm sieve, pooled to obtain one composite sample per plot, and air-dried.

Crop yields and carbon inputs. Crops were controlled during the two growing seasons of the study (year 1 and year 2). Carbon inputs were calculated in four steps. First, the harvest index (HI) was determined as the ratio of crops grain yield and aboveground biomass (AGB) from crop samples taken before harvest.

Second, total AGB in each plot was calculated using HI and the measured grain yield at harvest. Third, belowground biomass was estimated using empirical allometric relations given by IPCC (2006), as a percentage of AGB (24 % in wheat, 22 % in maize). Finally, the C concentration in each crop was used to translate mass into organic C data (see below).

Total organic C and C isotope analyses. Total organic C and the natural abundance of ^{13}C were analyzed in soil samples and crop compound samples (leaves, stalks and cobs) in a CHN elemental analyzer (NC 2500, Carlo Erba, Milano, Italy) coupled to a mass spectrometer (Thermo Quest-Finningan Delta Plus, Bremen, Germany). Acid fumigation (Harris et al., 2001), which has been observed not to affect the organic C concentration or isotopic signature in previous studies (Fernández-Ugalde et al., 2011), was used before analysis for soil carbonates removal. The SOC stock was calculated using SOC concentrations and bulk density data for an equivalent dry soil mass of 141 kg m^{-2} in all treatments (corresponding to the soil mass of the treatment with the lowest bulk density (M-irr) in the studied depth (Ellert & Bettany, 1995)).

The incorporation of maize-C into the soil was quantified in the M-irr and M-rf from the ^{13}C isotopic signature of SOC, expressed in $\delta^{13}C$ against the PBD international standard. The proportion of maize-derived C (*f*) in bulk soil samples in year 2 was calculated using Equation 1 (Denef et al., 2001):

$$f = \frac{\delta_{sample,y2} - \delta_{sample,y0}}{\delta_{maize} - \delta_{sample.y0}} \quad \text{Eq [1]}$$

where $\delta_{sample,y2}$ is the $\delta^{13}C$ of the studied sample in year 2, $\delta_{sample,y0}$ is the $\delta^{13}C$ of the soil sample at time 0 (year 0), and δ_{maize} is the average $\delta^{13}C$ of maize plant material (-12.93±0.1‰ in irrigated maize and -13.74±0.2‰ in rainfed maize).

Aggregate-size fractionation and C distribution. Soil samples from year 0 and year 2 were fractionated by size with a protocol designed after the hierarchical model of aggregation (Six et al., 2002) as in Fernández-Ugalde et al. (2011). Soil samples <5 mm were wet-sieved to obtain large macroaggregates (2000-5000 µm), small macroaggregates (250-2000 µm), microaggregates (50-250 µm), and the silt + clay fraction (<50 µm). The relative abundance of the each fraction was calculated as their weight proportion in the total original sample. The concentration of total organic C and ^{13}C as well as the proportion of maize-derived C (*f*) in each aggregate fraction in year 2 was analyzed and calculated as explained above. In the small macroaggregates and microaggregates fractions, sand contents were determined after complete dispersion trough sonication (Fernández-Ugalde et al., 2011), for correction of the aggregate weight proportion and the organic C concentration, to minimize the effect of sand dilution on C content (Six et al., 2002). This was not done for large macroaggregates (>2000) due to the small quantity of this fraction obtained (<1 % of the total soil mass).

Statistical analysis. The interaction of time and treatments was verified for all variables compared in time and among treatments in multivariate analysis. Then, factorial ANOVA analyses were run to

compare the different treatments, and the separation of mean values was performed using Duncan's test with a significance level of *P= 0.05* unless otherwise indicated.

RESULTS

Grain yield and C inputs. Yield was higher in M-irr than M-rf during both years and in W-irr than W-rf in year 1 (Table 2). In W-irr and W-rf, yields were similar in year 2 and smaller than in year 1 because climatic conditions (water excess) led to a delay in sowing (December 16). Differences in C inputs among treatments were observed only in year 1 between W-irr and W-rf, and irrigation had no effect on the cumulative C inputs for the 2 years in either crop (M-irr vs M-rf and W-irr vs W-rf) as a result of different HI values for each crop and management (Table 2). M-irr and M-rf produced almost twice the amount of cumulative C inputs than W-irr and W-rf (Table 2).

Table 2. Crop yields, harvest index and C inputs from crop biomass in year 1 and year 2 for wheat and maize in the two cropping systems.

		Wheat		Maize	
		Irrigated	**Rainfed**	**Irrigated**	**Rainfed**
Yield	Year 1	7.4 (0.1) b	5.3 (0.2) d	8.5 (0.2) a	6.0 (0.2) c
(Mg ha^{-1})	Year 2	4.4 (0.3) c	4.1 (0.1) c	14.4 (0.4)a	7.7 (0.6) b
Harvest	Year 1	0.46(0.01)a	0.45 (0.02)a	0.57(0.02)b	0.50(0.03)a
Index	Year 2	0.39 (0.01)b	0.33 (0.01)a	0.54(0.02)d	0.48(0.03)c
Residue	Year 1	2.1 (0.1) b	1.7 (0.1) c	2.8 (0.2) a	2.9 (0.3) a
biomass	Year 2	1.5 (0.1) b	1.6 (0.1) b	5.1 (0.3) a	3.8 (0.6) a
C(Mg ha^{-1})	Cumulative	3.6 (0.3) b	3.3 (0.1) b	7.9 (0.6) a	6.6 (0.9) a

Mean values and standard error (in brackets).
Values followed by different letters within the same row are significantly different at *P<0.05*.

Organic C storage and maize-C in soil. The total organic C stock in year 2 showed no difference compared with the baseline at year 0 in any treatment (Table 3). Bulk soil $\delta^{13}C$ values in year 0 were homogeneous among treatments (*P* = 0.608) and averaged -26.3±0.2 ‰ for bulk soil (Table 4). In year 2, the isotopic signature of SOC increased in M-irr and M-rf treatments. The effect was significantly higher in M-irr than M-rf (Table 4).

The proportion of SOC from maize biomass (Equation 1) was greater in M-irr than in M-rf (13.43 ± 0.69 % and 4.94 ± 1.37 %, respectively).

The mass of maize-derived organic C present in M-irr and M-rf in the studied depth was calculated from total organic C data. Then, using the cumulative values of organic C added to the soil as maize biomass in year 1 plus year 2 (Table 2), the proportion of this organic C incorporated into the soil was calculated. This proportion was significantly greater in M-irr (than in M-rf (0.34 ± 0.03 and 0.16 ± 0.04 kg maize-C incorporated per kg of maize biomass C added to the soil, respectively).

Aggregation and maize-C incorporation in aggregate fractions. Results of the fractionation protocol were satisfactory in terms of mass recovery (average recovery 97.3 ± 0.3 %), and organic C recovery obtained after C stock calculations (average recovery 97.6 ± 2.1 %). Aggregation was first evaluated in all plots at year 0. The soil aggregate-size distribution did not show significant differences among the four treatments (P > 0.05 for all fractions; Figure 2). Microaggregates accounted for near 50 % of the soil sand-corrected total mass. Small macroaggregates and the silt + clay fraction represented around 25 % each. Large macroaggregates were less than 1 % in all treatments. In year 2, the only two fractions showing changes in relation with year 0 (baseline) were large macroaggregates and silt + clay. Large macroaggregates in W-irr and M-irr augmented (from 0.20 to 1.27 % and from 0.24 to 0.81 %, respectively), and an increase occurred in the silt + clay fraction in three of the treatments (W-irr from 27.4 to 32.6 %, W-rf from 25.6 to 34.7 % and M-rf from 28.2 to 32.9 %).

Some differences were observed in organic C storage in aggregates size-fractions with time (Table 3). The stock in the large macroaggregates fraction augmented in year 2 in W-irr and M-irr plots. The interaction of time and treatment was also significant for this fraction. Increases in the stock were also observed in the silt + clay fraction for all treatments except W-irr. Small macroaggregates showed changes in M-rf, and microaggregates did so only in W-rf. As for bulk soil, $\delta^{13}C$ in aggregates size-fractions in year 0 were homogeneous among treatments (P > 0.05 in all fractions, Table 4), and averaged -27.8 ± 0.3 ‰ for large macroaggregates, -26.5 ± 0.2 ‰ for small macroaggregates, -26.0±0.1 ‰ for microaggregates and

-26.1±0.2 ‰ for silt+clay. After two years in the new cropping conditions, M-irr showed higher $\delta^{13}C$ values than in year 0 in large macroaggregates, small macroaggregates and microaggregates fractions. The absolute values of these differences decreased with aggregate size. Large macroaggregates in M-rf also showed higher $\delta^{13}C$ values compared with year 0, but no differences were observed in the rest of the fractions.

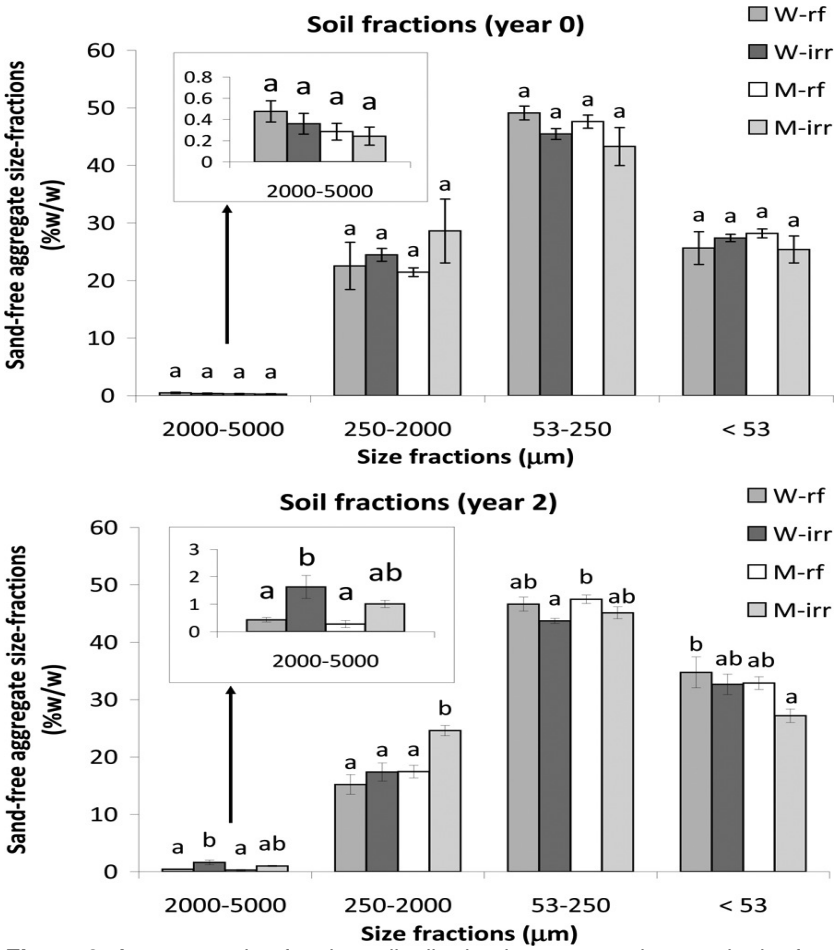

Figure 2. Aggregate size-fractions distribution in year 0 and year 2 in the four different treatments: W-rf (rainfed wheat), W-irr (irrigated wheat), M-rf (rainfed maize) and M-irr (irrigated maize).
Bars represent the standard error for each fraction and treatment. In year 2, letters within the same fraction indicate significant homogeneous groups among treatments at *P<0.05*.

Table 3. Soil organic carbon stock (Mg C ha^{-1}) in an equivalent mass (141 kg m^{-2}). Evolution from year 0 to year 2 in four experimental treatments.
Mean values and standard error (in brackets).

		Wheat		Maize	
		Irrigated	**Rainfed**	**Irrigated**	**Rainfed**
Bulk soil	Year 0	19.9 (1.48)	18.2 (2.45)	17.9 (0.94)	18.7 (0.60)
	Year 2	20.4 (0 .94) ab	21.6 (0.77) a	17.9 (0.40) c	18.4 (0.18) bc
Soil fractions					
Large macroaggregates	Year 0	0.23* (0.06)	0.30 (0.07)	0.15* (0.06)	0.18 (0.05)
(2000 – 5000 μm)	Year 2	0.98* (0.26) ab	0.39 (0.07) b	1.14* (0.15) a	0.48 (0.23) b
Small macroaggregates	Year 0	5.47 (0.03)	4.95 (0.83)	4.96 (0.61)	4.71* (0.36)
(250 – 2000 μm)	Year 2	5.37 (0.88)	5.03 (0.56)	4.51 (0.26)	3.86* (0.13)
Microaggregates	Year 0	8.32 (0.23)	8.47* (0.43)	7.76 (0.82)	9.85 (0.21)
(50 – 250 μm)	Year 2	8.40 (0.35) c	10.8* (0.29) a	8.57 (0.11) c	9.91 (0.24) b
Silt + clay fraction	Year 0	4.27 (0.13) a	3.35* (0.24) b	3.34* (0.28) b	4.46* (0.21) a
(< 50 μm)	Year 2	4.14 (0.16) b	5.31* (0.33) a	4.31* (0.06) b	5.27* (0.12) a

For each treatment and fraction, values followed by * indicate significant differences between year 0 and year 2 at *P<0.05*.
Values followed by different letters within the same row for each year are significantly different at *P<0.05*.

Table 4. Organic carbon $\delta^{13}C$ value (‰). Evolution from year 0 to year 2 in four experimental treatments. Mean values and standard error (in brackets).

		Wheat		Maize	
		Irrigated	**Rainfed**	**Irrigated**	**Rainfed**
Bulk soil	Year 0	-25.9 (0.5)	-26.8 (0.7)	-26.3* (0.3)	-26.2$^{\#}$ (0.1)
	Year 2	-26.2 (0.2) b	-26.1 (0.2) b	-24.5* (0.1) a	-25.7$^{\#}$ (0.2) b
Soil fractions					
Large macroaggregates	Year 0	-28.7 (0.6)	-27.4 (0.6)	-27.8* (0.4)	-27.4* (0.4)
(2000 – 5000 µm)	Year 2	-27.7 (0.1) c	-27.6 (0.1) c	-16.0* (1.1) a	-22.7* (0.2) b
Small macroaggregates	Year 0	-26.9 (0.3)	-26.6 (0.4)	-26.2* (0.1)	-26.6 (0.4)
(250 – 2000 µm)	Year 2	-26.8 (0.1) b	-26.6 (0.1) b	-25.1* (0.2) a	-26.4 (0.1) b
Microaggregates	Year 0	-25.9 (0.1)	-26.1 (0.1)	-26.1* (0.0)	-26.1 (0.1)
(50 – 250 µm)	Year 2	-26.3 (0.1) b	-26.3 (0.1) b	-25.4* (0.3) a	-26.1 (0.2) b
Silt + clay fraction	Year 0	-26.7 (0.6)	-26.1 (0.1)	-25.8 (0.0)	-25.9 (0.1)
(< 50 µm)	Year 2	-25.9 (0.1)	-25.9 (0.1)	-25.6 (0.1)	-25.7 (0.4)

For each treatment and fraction, values followed by * indicate significant differences between year 0 and year 2 at *P<0.05*. Values followed by $^{\#}$ indicate significant differences between years at *P<0.10*.

Values followed by different letters within the same fraction and year are significantly among treatments at *P<0.05*.

Finally, the isotopic signature of SOC in the silt + clay fraction was homogeneous among all treatments in year 0 and year 2. As a result, greater percentages of maize-derived organic C were found in M-irr than M-rf in large macroaggregates and small macroaggregates (Figure 3). The greatest proportion of maize-derived organic C was incorporated for both treatments in large macroaggregates.

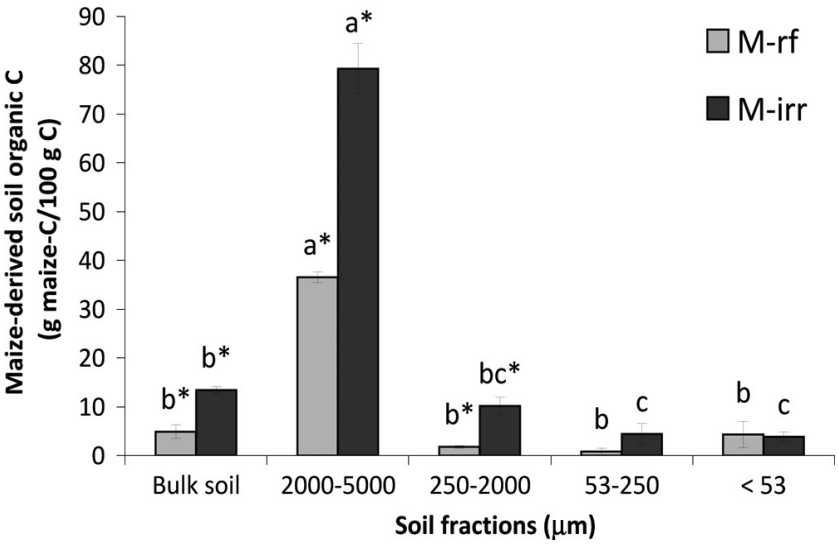

Figure 3. Proportion (%) of maize-derived organic C in year 2 in bulk soil and aggregate size-fractions of maize plots: M-rf (rainfed maize) and M-irr (irrigated maize).
Bars represent the standard error for each fraction and treatment. Significant differences (*P<0.05*) between treatments for each fraction are marked with *. Letters within the same treatment indicate significant differences among fractions at *P<0.05*.

DISCUSSION

C inputs and soil organic C stocks. It was hypothesized in this study that the changes in the agroecosystem associated to the adoption of irrigated agriculture, would lead to different grain yields and different crop biomass production available to be incorporated into the soil than in rainfed systems (Figure 1). Differences between crops were indeed clear in terms of yield (except for W-irr and W-rf in year 2, Table 2). However, for each crop, irrigation did not increase C inputs on average in the two years of the study (Table 2). In addition,

no differences in total SOC stocks were observed in time for any treatment (Table 3), unlike hypothesized following previous studies showing an increment in SOC stocks due to increased biomass inputs (Adviento-Borbe et al., 2007) and the implementation of irrigation (Gillabel et al., 2007; Denef et al., 2008).

The relatively short period considered (2 years) can explain the lack of significant changes in SOC stocks. Additionally, an explanation for the lack of differences in residue biomass C after two years in irrigated and rainfed wheat and maize can arise from the fact that the HI was different and displayed higher values in the irrigated plots on average for both crops. This means that under irrigated conditions, grain accounted for a greater proportion of the total crop biomass. Some studies have reported similar results (Giaveno et al., 2002) due to the fact that modern varieties prioritize a bigger partitioning of assimilates to the fruit, so a higher increase in grain yield than aboveground biomass occur when the cropping conditions are improved. In addition, the potential effect of irrigation to increase SOM mineralization (Sainju et al., 2008; Jabro et al., 2008) due to changes in the soil moisture regime and microorganisms activity, could have counteracted the slightly greater inputs observed in W-irr than in W-rf in year 1 and M-irr than M-rf in year 2 (Table 1), resulting in an overall nil net effect of irrigation on SOC stocks in the studied 2-years period (Figure 1).

Organic matter dynamics in maize plots. Significant changes were observed in the isotopic signature of soil C in maize plots (M-irr and M-rf) from year 1 and year 2 (Table 4). The activation of the soil biological activity, and other processes related to organic matter mixing during soil management, can result in changes in the ^{13}C concentration of the soil organic fraction (Wynn et al., 2006). In the studied plots, the absence of differences in $\delta^{13}C$ values with time in W-rf and W-irr (Table 4) indicates that the observed changes in M-irr and M-rf can be attributed to the incorporation of maize residues, and not to changes induced by irrigation itself or to other changes in management. Other studies analyzing C3-to-C4 crops transitions have also observed changes in the isotopic signature of the soil organic fraction in relatively short periods of time (e.g. Urbanek et al.,

2011). In addition, results on the proportion of maize-C indicated that the incorporation of crop residues was different in M-irr than in M-rf (Table 4 and Figure 3). Although total SOC stocks were similar in M-rf and M-irr (Table 3), the proportion of maize C found in the bulk soil in M-irr was greatly higher than in M-rf. This was also true when considering the proportion of total maize residues incorporated into the soil in each treatment. These results confirm that the turnover of maize residues and SOC were accelerated in the studied depth in the irrigated treatment (M-irr), despite of the total SOC stock not changing significantly with time or in relation to the non-irrigated treatment (M-rf). This observation confirms those of others (Gillabel et al., 2007; Denef et al., 2008) that irrigation can effectively modify the incorporation cycle of organic matter into the soil, by activating the mechanisms favoring both its incorporation and mineralization (Figure 1). In a recent study, Follett et al. (2013) compared the results of SOC accumulation under no-tillage (NT) in irrigated maize, with a previous study (Follett et al., 2012) in rainfed maize. They observed no accumulation of extra SOC after 9 years of NT in the irrigated plots, for a net increment in dryland conditions (Follett et al., 2012). They attributed this difference to different climate and crops conditions, but also to the observed loss of old carbon (C3-C) due to enhanced decomposition of this C pool under irrigation. Our results support their hypothesis that irrigation activates not only the incorporation of fresh crop residues, but also the mineralization of older SOC. Previous studies have shown indeed an organic matter mineralization rise with irrigation or re-wetting, expressed as an increase in the CO_2 emission rate (Miller et al., 2005; Sainju et al., 2008; Jabro et al., 2008) related to the increase of the activity of decomposer microorganisms, which can counteract the effect of greater organic inputs (Figure 1), resulting in no net gains of SOC (Verma et al., 2005; De Bona et al., 2008). In agroecosystems where the soil is less disturbed than extensive cropland, such as grazed pastures, it has also been observed that the turnover of root C is accelerated when irrigation is applied (Scott et al., 2012), resulting in some cases in SOC losses in the long-term compared to non-irrigated plots with lower primary productivity and stocking rates (Kelliher et al., 2012).

Aggregation and soil organic C distribution. The results obtained in this study showed a slight evolution in soil aggregation overall, despite the differences observed between M-rf and M-irr in organic matter incorporation. In Figure 2 several differences among treatments can be seen in year 2, but the only relevant evolution produced from year 0 to year 2 was the increase of the proportion of large macroaggregates, affecting both irrigated treatments (M-irr and W-irr). In relation to our hypothesis, this increment supports the idea of changes in aggregation in irrigated systems (Figure 1). However, this fraction represented only around 1 % of the total soil mass, which means that this cannot be used to extract general conclusions on aggregation dynamics in relation to the different treatments studied. In addition to the short time-span considered in this study, two reasons can explain this small evolution in aggregation. On one hand, the intensive management of the experimental plots could avoid or reduce macroaggregates stabilization (Blanco-Canqui & Lal, 2004), as represented in Figure 1. On the other hand, the relationship between soil organic matter and aggregation is known to be weaker in carbonate-rich soils (Abiven et al., 2009) as the one studied here (carbonates accounted for 36 % of the total soil mass in the studied depth). In relation to the distribution of organic C among aggregates size-fractions (Table 3), the observed differences correspond to those observed in the aggregates-size distribution (Figure 2). In relation to maize-C incorporation, large macroaggregates and small macroaggregates had higher proportions of maize C in M-irr than in M-rf (Figure 3). Following the hierarchical model of aggregation (Tisdall and Oades, 1982; Golchin et al., 1994; Six et al., 2000), fresh organic matter from crop residues activates the aggregation cycle (Figure 1) and is first incorporated in large aggregates, providing them with stability and creating the hotspots were smaller aggregates form within the larger ones. As the metabolization of these residues proceeds, large aggregates lose their stability and organic matter from crop residues can be found within the smaller aggregates, which in turn will constitute the basic units to new macroaggregates formed around newly arrived crop residues. The observation of significant but decreasing proportions of maize-C in small macroaggregates and microaggregates than in large macroaggregates in M-irr (Figure 3)

indicates that some type of transfer existed among aggregate fractions in this soil when irrigation was introduced, following the schema shown in Figure 1, which were much less evident in the rainfed treatment. However the above-mentioned factors indicate a weak relationship between organic matter turnover and aggregation in the studied soil.

CONCLUSIONS

This study was specially designed to observe the effect the introduction of irrigated crops in dryland areas. Unlike hypothesized, no significant differences were observed in the cumulative crop biomass potentially incorporable to the soil in the two first years after the conversion from dryland to irrigation. As a result of this, and very likely because of the short time lapse considered, the overall SOC stock did not change among the studied treatments after two years since the starting of the experiment. Evidence was however found of an influence of irrigation adoption on the incorporation pace of maize residues in the soil. As hypothesized, incorporation rates were greater in the irrigated plots, and implied a more active transfer of crop residues into smaller aggregate sizes than in the rainfed maize agroecosystem. These results support the idea of irrigation changing the incorporation and mineralization dynamics of crop residues into the soil organic pool, and indicate that the introduction of irrigated and new crop management systems in dryland semiarid areas can accelerate soil organic C dynamics before observable changes in organic C stocks soil aggregation appear. The potential consequences of these changes should be considered when quantifying the impact of the adoption of irrigation in soil quality and soil C sequestration in semiarid land. Soil quality is understood as the capacity of a soil to exert its functions within the limits of the agroecosystem, such as sustaining the productivity of plants and animals and maintaining water quality (Karlen, 1997). Our results indicate that the relationship between crop yields and the benefits of crops residues incorporation into the soil, and thus the potential delivery of nutrients from organic matter decomposition and the development of soil structure, are likely to be different in dryland and

irrigated agroecosystems. In relation to organic C sequestration, the different pace of crop residues incorporation into the soil in irrigated and dryland soils indicate that the proportion of atmospheric C from crops stabilized in the soil is also affected by this change. This needs to be accounted for in C balances and GHG gases inventories of irrigated agroecosystems.

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Chapter III

Role of carbonates in soil organic matter stabilization

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ABSTRACT

Carbonated soils are present in many semiarid areas, where lithogenic and secondary carbonates are important constituents of the soil mineral matrix. The presence of $CaCO_3$ in calcareous soils has been described as an organic matter stabilization agent mainly due to chemical stabilization mechanisms. In two recent studies in the north of Spain the importance of $CaCO_3$ on soil physical characteristics has been highlighted, as they were observed to be acting as macroaggregates stabilization agents. The present study has been carried out in the same site, with the hypothesis that the observed differences in aggregation may favor organic matter stabilization in carbonate-containing soils. With that aim we have studied the soil physical characteristics (water retention and porosity) and the bioavailability of soil organic matter (SOM) in the two contrasting soils in that site, Typic calcixerept (CALC) and Calcic Haploxerept (DECALC). Bioavailability was evaluated trough the measurement of mineralization rates in a 30 days soil incubations. Intact and disaggregated samples were incubated to evaluate the effect of physical protection on SOM bioavailability in whole soil and macroaggregates 2-5 mm samples. Therefore, four fractions of each soil were studied: intact whole soil < 5 mm (I-WS), disaggregated whole soil (D-WS), intact macroaggregates 2-5 mm (I-Magg), and disaggregated macroaggregates (D-Magg). Soil organic carbon content was greater in CALC and had smaller mineralization rates during incubation, indicating a smaller organic matter bioavailability for microbial decomposition. However, the greater increment of mineralization observed in DECALC after disaggregation, together with the scarce differences observed in physical characteristics among both soils; indicate that physical protection was not responsible of greater SOM stability in CALC soil.

INTRODUCTION

The importance of the soil mineral matrix for SOM stabilization. Soil organic matter is of special importance in soil quality and functioning due to its relevance in physical, chemical and biological processes. It has influence in the structure development, microbial activity and biodiversity, available water capacity, root depth and nutrient cycle (Wander et al., 2004). For this reason, several authors have proposed soil organic matter as an appropriate indicator of soil quality (e.g. Karlen et al., 1997; Lal, 1998; Stenberg, 1998; Sands & Podmore, 2000; Arshad & Martin, 2002).This is more remarkable in soils depleted in organic matter such as arable soils in the Mediterranean area, where the low soil organic carbon content may indicate soil degradation (Romanya & Rovira, 2011).

The organic matter being part of a dynamic cycle, its content is determined by the initial content, the organic matter inputs and organic matter losses (Paustian et al., 1997; West & Post, 2002). Mineralization is the main mechanism responsible for organic matter losses, and implies CO_2 emission to the atmosphere (Jackson et al., 2003; Martens et al., 2005). Stabilization mechanisms reduce organic matter losses, slowing down the cycle and promoting organic matter accumulation if all other parameters remain constant.

Biochemical recalcitrance of organic molecules, physical protection against decomposers, and chemical stabilization by interaction with the mineral matrix have been thoroughly recognized in the literature as the main stabilization mechanisms of organic matter in the soil (Six et al., 2002; Lützow et al., 2006; Jastrow et al., 2007; Kögel-knabner et al., 2008). Despite of this general agreement there are many factors and processes involved and acting at the same time, which makes it difficult to estimate the relevance of each mechanism separately (Lützow et al., 2006). Moreover, important differences may occur among soils types and horizons.

Recalcitrance of the organic compounds has been deeply studied and proposed by some authors as the main mechanism involved in organic matter stabilization (Krull et al., 2003). Molecular properties that influence the decomposition rates of natural substrates are

molecule size, polarity, ether-bridges, quaternary C-atoms links, three-fold substituted N-linkages, phenyl- and heterocyclic N-groups as well as long-chain (hydrophobic) hydrocarbons (Lützow et al., 2006). However, scientific evidence is showing that recalcitrance is a dynamic process and is relevant mainly in surface soils for the initial phases of decomposition (Six et al., 2002; Lützow et al., 2006).

Several physical protection mechanisms (occlusion of organic matter by aggregation, interaction with phyllosilicates, hydrophobicity and encapsulation in organic macromolecules), and chemical stabilization mechanisms (ligand exchange, polyvalent cation bridges, weak interactions and complexation of metal ions) have been described (Lützow et al., 2006). These mechanisms are highly influenced by the soil physical-chemical properties. Thus, its presence or importance for organic matter stabilization will depend on the soil type.

Clay content and clay mineralogy are known to be determinant factors in organic matter stabilization. Clay content has a direct relationship with the amount of organic carbon associated with primary organomineral complexes (Hassink, 1997). Also clay characteristics determine the amount of organic carbon stabilized in soils. The specific surface area (SSA) controls the extent to which microbial metabolites are stabilized (Seggar et al., 1996), and differences in mean residence times among semectite and kaolinite dominated soils has been attributed to the different cation exchange capacities (Wattel-Koekkoek & Burrman, 2004). Therefore, clay mineralogy is supposed to be determinant in organic matter stabilization (Abiven et al., 2009). Moreover clay content and mineralogy are important factors for aggregation (Denef & six, 2005; Denef & six, 2006), favoring in an indirect way the stabilization of organic matter within aggregates (Denef et al., 2004).

Other physical-chemical properties such as texture, pH and the presence of polyvalent cations play an important role in determining the stabilization mechanisms of the organic matter in soils. Texture highly determines soil porosity and thus, has a big influence on the accessibility of microorganism to organic matter (Chenu & Stotzky, 2002). Ligand exchange has been observed to increase with

decreasing pH, with a maximal sorption between pH 4.3 and 4.7 (Gu et al., 1994). In addition, some organic groups (carboxyl and phenolic OH), which participate in organomineral unions, are only present in acidic soils. Finally, polyvalent cations (Fe^{3+}, Al^{3+}, Ca^{2+}, Mg^{2+}) influence stabilization mechanisms by participating in strong organo-mineral associations, in complexation processes of the organic molecules, and forming bridges between negative charged clay and negative charged organic compounds. So they have been considered stabilizing agents of the organic matter (Baldock & Skjemstad, 2000), although more research is needed to quantify their role.

Chemical and physical characteristics of carbonate-rich soils. Carbonated soils are present in many semiarid areas, where lithogenic and secondary carbonates are important constituents of the soil mineral matrix. These soils are characterized by an alkaline pH ranging from 7 to 8.4, and an exchange complex dominated by Ca^{2+}. High values of cation exchange capacity can be frequent in these soils, because of the increase of negative pH dependent charges in organic matter compounds and clay with high levels of pH (Brady and Weil, 2002). These chemical properties have consequences in the nutrient availability and the reactivity of the soil matrix. Several nutrient deficiencies are well known to occur in carbonated soils. The most relevant are deficiency of phosphorous, tied up in insoluble calcium, and deficiencies in micronutrient metals as iron and zinc (Brady and Weil, 2002). In relation with the reactivity of the soil matrix, the presence of Ca^{2+}multivalent cations favors cation bridging. That may promote mineral-mineral bonds among clay minerals (Six et al., 2004), inter-molecular interactions between the organic and inorganic soil components (Baldock and skjemstad, 2000), and also interactions among organic polymers (Ojeda et al., 2003).

The presence of carbonates in soils has also influence in the physical properties. Carbonates can strongly affect soil aggregates formation and stabilization (Munner and Oades 1989, six et al., 2004, bronick and lal 2005.) As a consequence, aggregates stability in these soils may not be related to OM quantity or quality (Boix-Fayos et al., 2001; Six et al., 2004; Bouajila and Gallali, 2008). However, results of several studies (Ojeda et al., 2003; Virto et al., 2007; Álvaro-Fuentes

et al., 2009) suggest a link between macroaggregate turnover and OC stabilization. These authors reported increments in the OM content following the application of agricultural practices as no-till or organic amendments, which have shown to be associated to enhanced macroaggregates stability. Thus, the aggregates dynamics in these soils and the role that carbonates are playing is still not clear. In a recent study, Fernández-Ugalde et al. (2011) tested for carbonated soils the applicability of the hierarchical model of aggregation, accepted for temperate soils where organic matter is the main stabilizing agent (Tisdall and Oades, 1982; Six et al., 1999; Six et al., 2004). The results showed that aggregation is stimulated in carbonated soils by the entrance of fresh organic matter. However, contrasting with the hierarchical model, aggregation showed a small dependence of organic matter decomposition. Thus, a coexistence of biotic and abiotic mechanisms of macroaggregates stabilization, with carbonates as rate-modifiers of the macroaggregates cycle, was postulated (Fernández-Ugalde et al., 2011). In a subsequent study the mechanisms of macroaggregate stabilization by carbonates were analyzed. Fernández-Ugalde et al. (2014) observed that the dissolution and reprecipitation processes of secondary carbonates are likely to be responsible of the greater stability of macroaggrgates in calcareous soils. The role of Ca^{2+} cation bridges remained unclear. Moreover macroaggregates formed in calcareous soils showed different physical characteristics than in similar soils without carbonates; with a more massive intra-aggregate fabric, smaller proportion of microaggregates within the macroaggregates, and a smaller intra-aggregates porosity proportion (Virto et al., 2013). These differences in physical characteristics of macroaggregates are likely to be related to the presence of abundant calcite crystals (Fernández-Ugalde et al., 2014).

Organic matter protection in carbonate-rich soils. Traditionally the presence of $CaCO_3$ in calcareous soils has been described as an organic matter stabilization agent. Duchafour (1976) suggested that the presence of reactive $CaCO_3$ can lead to the biological stabilization of organic matter. He proposed two mechanisms of stabilization, the formation of $CaCO_3$ coatings around fresh organic matter and the

presence of Ca-organic compounds linkage in humus. Afterwards, comparisons among different types of soils indicated that the presence of $CaCO_3$ leads to the accumulation of organic carbon. For instance, Oades (1988) observed greater organic carbon contents in calcareous sands in comparison with siliceous sands.

Baldock and Skjemstad (2000), in a review about the role of the mineral matrix in organic matter stabilization described the importance of Ca^{2+} cations in organic matter stabilization. They pointed out the influence of chemical mechanisms as suggested in Duchafour (1976). But they described as well the architecture of the soil matrix as an important factor in stabilization. That is due to its influence in the biological stability of organic materials, through its effects on water and oxygen availability, entrapment and isolation of organic materials from decomposer organisms, and the dynamics of soil aggregation. In accordance with the idea of protection regulated by the architecture of the soil mineral matrix, a more recent publication (Kemmit et al., 2008) proposed that a first, rate-limiting step of mineralization of organic matter is governed by abiotic processes termed “regulatory gate” processes, rather than microbial processes. In this first step organic matter mineralization may depend on physical characteristics such as texture, porosity or/and water availability. These properties can be modified by the presence of carbonates. As described by Falsone et al. (2010) and Catoni et al. (2012), the precipitation of carbonates decreases the soil macroporosity. Besides, the activation of carbonates precipitation after fresh organic matter addition to soil has been observed (Bertrand et al., 2007; Rovira and Vallejo, 2008). In agreement with the regulatory gate idea, recent studies developed on the soil used for the present study, showed that calcareous soil has a different aggregation dynamics (Fernández-Ugalde et al., 2011), leading to a different fabric of macroaggregates, which are more massive and have a less porous structure in calcareous soils. This may favor organic matter stabilization in these soils (Fernández-Ugalde et al., 2014).

The objective of this work was to study the different physical properties of the soil and macroaggregates matrix in carbonated and decarbonated semi-arid Mediterranean soils, and to determine the

consequences of these physical properties in the bioavailability of organic matter. Based on previous knowledge we hypothesized that the presence of carbonates results in a soil fabric that limits organic matter biodegradation, in comparison with soils without carbonates. We approached this study by comparing the behaviour of two similar soils differing in the presence of carbonates (used in Fernández-Ugalde et al., 2011) in terms of organic matter mineralization following different degrees of disaggregation.

Materials and Methods

Site description and sampling. This study was conducted on samples from two different soils within the same experimental plot (2 ha in total). The two soils differ mostly in their carbonates content, and have had the same management since at least 1954. Both soils are located in Rodezno, La Rioja (north of Spain). The calcareous soil is a Typic Calcixerept (Soil Survey Staff, 2006) with 20.9% clays and 22.1% carbonates, mainly accumulated in the clay-sized fraction. After carbonates removal, its proportion of clay decreased to 10.4%. The second soil from Rodezno is classified as a Calcic Haploxerept (Soil Survey Staff, 2006) with 21.5% clays, which was only slightly reduced to 20.0% after removal of the different forms of Fe, and with no carbonates in the upper Ap horizon (Table 1). According to Arpón (2009), who classified this soil, before agricultural disturbance, this soil had a sequence of horizons corresponding to a decarbonated *terra rosa* (Palexeralf: A, Bt, petrocalcic). Continuous agricultural management has resulted in the mixing of materials in the A and Bt horizons, developing its present Ap-petrocalcic sequence. The two soils will be named hereafter calcareous soil (CALC), and decarbonated terra rosa (DECALC), respectively. The two soils have been cultivated with a rotation based on wheat (Triticum aestivum L.), pea (Pisum sativa L.), and sunflower (Helianthus annuus L.) for the last decades. Conventional soil management in the area includes annual mouldboard tillage (0–30 cm) with crop residues incorporation and seed-bed preparation with a cultivator. Sampling was done between cultivation and seeding in the two soils.

Soil blocks (20×20×20 cm) were collected from the Ap horizon in three replicate points separated by approx. 100 m at each site. After collection, samples were divided in two replicates. First replicates were gently passed through a 5 mm sieve to preserve soil structure. These samples were kept field-moist for the analysis of some physical properties and the incubation experiment (see below). The second replicates were air-dried and sieved (2 mm diameter), and then, were analyzed for basic soil parameters according to standard procedures (Carter, 1993). For more details see Fernández-Ugalde et al. (2011). Part of the field-moist samples < 5 mm were sieved using a 2 mm-mesh sieve to separate macroaggregates 2-5 mm in size for each subsample. These aggregates, together with whole < 5 mm soil samples, were used for the physical analysis and the incubation, with the aim of evaluating organic matter preservation within macroaggregates.

Undisturbed 100 cm^3 soil cores were also collected in each plot in triplicate at the same locations, depths (0-20 cm) and time as the soil blocks. Soil cores were weighted field moist and after being oven dried at 105 ºC for bulk density (ρ_b) and soil moisture determination. Total porosity of each sample was calculated from bulk density and soil particle density, which was assumed to be 2.65 g cm^{-3}. Undisturbed soil cores were also used to measure soil water retention at matric potentials of -10 and -33 kPa (see below).

Table 1. Soil characteristics in the 0-20 cm depth. Average ± standard error (n=3)

	Soil 1 (CALC)	**Soil 2 (DECALC)**
Soil type (SSS, 2006)	Typic Calcixerept	Calcic Haploxerept (Palexeralf)
PSD* (g kg^{-1})		
Sand	425 ± 9.5	446 ± 14.0
Silt	366 ± 12.8	338 ± 10.5
Clay	209 ± 3.5	215 ± 4.5
Bulk density (gcm^{-3})	1.58 ± 0.01	1.63 ± 0.05
Carbonates (g kg^{-1})	221 ± 5.5	-
Organic C (g kg^{-1})	10.3 ± 0.4	9.1 ± 0.2

*PSD: Particle size distribution. Sand: 50-2000 µm, Silt: 2-50 µm, Clay: <2 µm

Physical properties

Water retention curves. Soil water retention (SWR) was determine on undisturbed cores at -10 and -33 (field capacity) kPa, on whole-soil samples at -50, -150 and -1500 (wilting point) kPa, and on field-moist 2-5 mm aggregates at -10, -33, -50, -150 and -1500 kPa. SWR was determined in triplicate for each sample in 5 and 15 bar pressure plate extractors (Soil Moisture Equipment Corp., Santa Barbara, CA), as described by Dirksen (1999). Volumetric values for the SWR were calculated from the gravimetric measures using ρ_b.

As described in Bescansa et al. (2006) and Fernández-Ugalde et al. (2009), the model proposed by Rose (1966) was used to estimate the equivalent pore diameter corresponding to each of the water potentials. According to this model, equivalent pore diameter was of 34 μm for -10 kPa, 10.3 μm for -33 kPa, 6.8 μm for -50 KPa, 2.3 μm for -150 KPa, and 0.2 mm for -1500 kPa. The conversion of SWR data at different potentials into different equivalent pore-size ranges was used to compare the porosity < 34 μm in the two soils and macroaggregates.

Micromorphology. Thin sections 5.5 cm long, 4.5 cm wide, were prepared from undisturbed soil cores and 2-5 mm aggregates, according to the methods described in Murphy (1986). Because of their nature, no special drying methods were required to maintain their original structure. For aggregates, five to eight individuals were selected and used to prepare these sections.

Thin sections were used to analyze the fabric and characteristics of macroaggregates, using an Olympus BX51 petrographic microscope coupled with a digital camera. The description was done following the guidelines given by Stoops (2003).

Image analysis was used to determine parameters related to macroaggregates porosity as in Virto et al. (2013) and Fernández-Ugalde et al (2014). Briefly, photographs (1000 x 750 μm^2) were taken on three spots per unaltered sample or per aggregate on three different aggregates per soil. Twin photographs (one under polarized light (PL) and another one using circular cross-polarized light (CPL) to avoid extinction phenomena in mineral grains) were taken at each

spot. On these photographs, image analysis was run using the free software Image Tool 3.0 (UTHSCSA, University of Texas Health Center in San Antonio), as described in Marcelino et al. (2007), in various consecutive steps. First, the two images were converted from color to grayscale, and then the PL image was subtracted to the CPL image. This produces an image in which all pores (resin) are black because they are the only features being black under CPL and white under PL. Organic matter and opaque bodies, which are black or dark in both images, appear white or grey. In a similar way, all other elements displaying some degree of color in CPL or PL pictures appear grey in the subtracted image.

Second, the subtracted image was used to obtain a black-and-white image in which only pores appear black (segmentation). This was done by determining a threshold value of grey intensity in the subtracted image above which all features corresponded to dark pores. The binary image was used to determine percent porosity and pores characteristics. The segmentation threshold, which is a crucial point for image analysis (Marcelino et al. 2007; Peth et al. 2008; Elyeznasni et al. 2012; Rasa et al. 2012) was determined manually for each image, to ensure appropriate separation of the pores volume and the soil matrix. This allowed to study the porosity for pores >20 μm, which is the lowest possible resolution using this system because it corresponds to the thickness of the sections.

Soil incubation. Carbon mineralization rates were measured using soil incubations with the aim of evaluating the bioavailability of SOM in both soils (CALC and DECALC), and their macroaggregates (2-5 mm) fraction. Intact and disaggregated samples were incubated to evaluate the effect of physical protection on SOM bioavailability. Therefore, four fractions of each soil were studied: intact whole soil < 5 mm (I-WS), disaggregated whole soil (D-WS), intact macroaggregates 2-5 mm (I-Magg), and disaggregated macroaggregates (D-Magg). Disaggregated samples were obtained by grinding them to pass through a 0.25 mm sieve.

Three samples (6 gr.) of each treatment were weighed and placed in plastic centrifuge tubes (50 ml, 3.5 cm diameter). Then samples were moistened to field capacity according to previous

measurements, and hermetically sealed. Finally they were introduced in an automatic low temperature incubator together with an empty tube used as blank. A two-step pre-incubation was performed, where tubes were opened and aerated after the first 24 hours of incubation to remove CO_2 flush generated by water addition, and headspace CO_2 was measured to ensure ambient air concentrations. As a second step, a 7-day pre-incubation was carried out at a constant temperature of 25 ºC to allow the Birch effect and other disturbance effects to pass, in which tubes were opened and aerated every 24 hours, and headspace CO_2 concentrations were measured.

Incubations were then continued for 30 days at a constant temperature of 25 ºC. Headspace gas samples were analyzed for CO_2 concentration, using a LICOR LI-6252 infrared gas analyzer (IRGA). Headspace sampling was done every 24 hours (days 1-7), then every 48 hours (days 8-15), and then every 72 hours (days 16-30). Tubes were aerated, when needed, to ensure that CO_2 concentrations did not exceed 20.000 ppm, to prevent CO_2 concentration from inhibiting microbial activity. Soil moisture was checked and corrected when needed after the jars were aerated.

A parallel experiment was run to determine the rates of soil CO_2 sequestration as carbonates precipitation and/or the emissions of abiotic CO_2 in CALC samples during the incubation. That for, an experiment using progressive enrichments of CO_2 in the atmosphere of tubes prepared as described above was run on sterilized samples from CALC and DECALC, following Oren & Steinberger (2008). No significant differences were observed in the amount of CO_2 precipitated as $CaCO_3$ between CALC and DECALC, because Ca was available in both soils (data not shown), and therefore total CO_2 present in the incubated tubes at sampling was assumed to correspond to total respired CO_2 in both soils during the experiment.

Statistical analysis. Data were analyzed using ANOVA (univariate linear model) to determine significant differences among soils and treatments. For each parameter, data were tested for different between soils and among treatments for each soil. Treatment means were compared, and post-hoc analysis was performed using Duncan's test. Significant results are based on a probability level of

P=0.05. All statistical analyses were performed using SPSS 22.0 software (SPSS Inc., 2013, Chicago IL).

Results

Water retention and porosity. Water retention at field capacity (-33 kPa) was only slightly different in both soils: CALC stored 0.29 ± 0.00 cm^3 cm^{-3} and DECALC stored 0.27 ± 0.01 cm^3 cm^{-3} (P = 0.058). Significant differences among both soils existed in macroaggregates (2-5 mm) for the water retention at field capacity (-33 KPa): CALC stored (0.30 ± 0.00 cm^3 cm^{-3}) and DECALC (0.27 ± 0.01 cm^3 cm^{-3}) (P=0.013). These values were used to moisten whole soil samples and aggregates to field capacity prior to the incubation experiment.

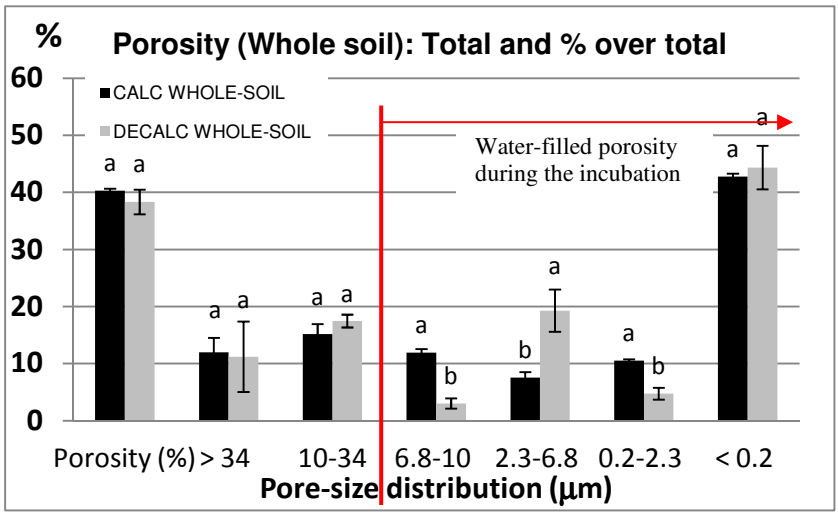

Figure1a. Total volumetric porosity and percentage over the total of each pore-size class in whole soil samples in Calc and Decalc soils. Different letters indicate significant differences among soils in each pore size class analyzed (P < 0.05). Errors bars represent standard errors (n = 3).

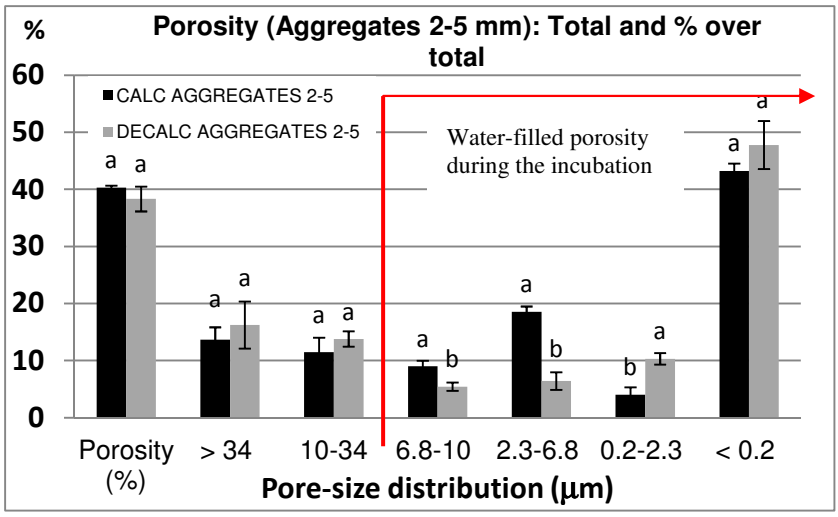

Figure1b. Total volumetric porosity and percentage over the total of each pore-size class in macroaggregates (2-5 mm) samples in Calc and Decalc soils. Different letters indicate significant differences among soils in each pore size class analyzed ($P < 0.05$). Errors bars represent standard errors ($n = 3$).

No differences in bulk density (data not shown) and total volumetric porosity were observed between CALC and DECALC. However, a different pore-size distribution was observed in the two soils. Differences existed in three pore size classes: 6.8-10 µm, 2.3-6.8 µm, and 0.2-2.3 µm (Figure 1a and 1b). In whole soil samples, CALC had a greater porosity in 6.8-10 µm and 0.2-2.3 µm pore size classes and smaller porosity than DECALC in 2.3-6.8 µm. The distribution of pores was different in macroagregates (2-5 mm), having CALC a greater proportion of their porosity than DECALC in 6.8-10 µm, 2.3-6.8 µm, and smaller in 0.2-2.3 µm. Assuming that pores smaller of 0.2 µm are too narrow for the accessibility of microorganism and pores above 10 µm were air filled at the matric potential (-33 kpa) of the incubation, the volume of pores accessible to microorganism was calculated as the sum of 6.8-10 µm, 2.3-6.8 µm, and 0.2-2.3 µm pore size classes. For whole soil samples there were no difference ($P=0.454$) in the calculated volume between CALC (30.0±0.3 %) and DECALC (27.1±3.6 %). For 2-5 mm macroaggregates CALC had a greater ($P=0.005$) volume percentage (31.6±1.1%) than DECALC (22.2±1.3 %).

Soil incubations. At the end of the incubation, the total accumulated respired CO_2-C was of 27.5 µg CO_2-C mg^{-1} soil OC in CALC-intact whole soil samples, significantly smaller (P=0.005) than 33.9 µg CO_2-C mg^{-1} soil OC observed DECALC-intact whole soil samples. For the disrupted samples, 30.8 µg CO_2-C mg^{-1} soil OC were respired in CALC-disrupted whole soil samples, for 46.7 µg CO_2-C mg^{-1} soil OC in DECLC-disrupted whole soil samples (P=0.061). In addition, significantly greater respiration occurred in disrupted than intact samples in DECALC (P=0.045), but not in CALC (P=0.481) (Figure 2).

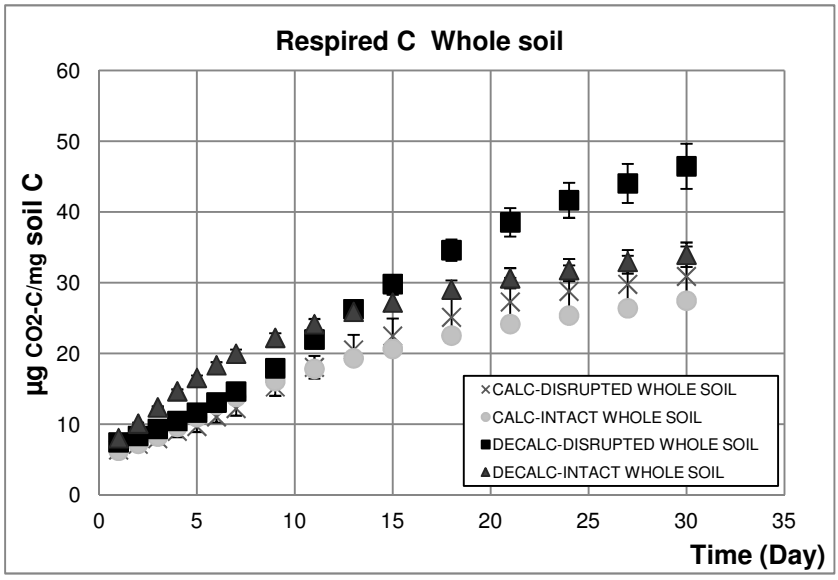

Figure2. Cumulative C respired (µg CO_2-C mg^{-1} soil OC) in 30 days incubation in Calc and Decalc whole soil samples, intact and physically disrupted. Errors bars represent standard errors (n = 3).

For macroaggregates (2-5 mm) samples, the accumulated respiration at the end of the incubation was of 27.2 µg CO_2-C mg^{-1} soil OC in CALC intact samples, not significantly different (P=0.324) from the 31.8 µg CO_2-C mg^{-1} soil OC in DECALC intact samples. Disrupted samples did not show greater accumulated respiration than the non-disrupted ones in CALC (P=0.108) and only slight differences in DECALC (P=0.076). In CALC disrupted macroaggregates, total respiration was of 41.6 µg CO_2-C mg^{-1} soil OC, not significantly

different (P =0.747) from DECALC disrupted macroaggregates (44.2 µg CO_2-C mg^{-1}).

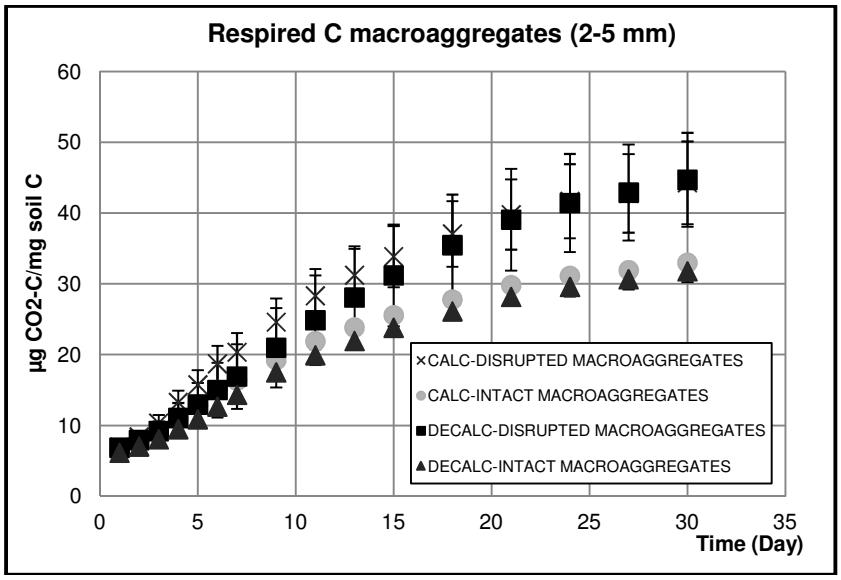

Figure 3. Cumulative C respired (µg CO_2-C mg^{-1} soil OC) in 30 days incubation in Calc and Decalc macroaggregates (2-5 mm) soil samples, intact and physically disrupted. Errors bars represent standard errors (n = 3).

Both soils showed a phase of increasing respiration rates, and a later decrease after reaching a peak of maximum respiration rates (Figure 4). This peak of respiration in DECALC intact samples was greatergreater than for CALC intact (P=0.047) and among DECALC disrupted and CALC disrupted peaks no differences were found (P=0.125). It is also noticeable that in the two soils a lag in mineralization rates existed in the disrupted samples in comparison with intact samples. The time-lapse of this lag between intact and disrupted peaks was longer in DECALC than in CALC (10 days vs. 3 days). Disrupted samples also displayed greater respiration rates than intact samples in DECALC until the end of the incubation (P=0.040 at day 30). In CALC this was true only until day 11 (P=0.05 at day 11; P = 0.16 at day 13). It has to be reminded that both soils had been pre-incubated, and therefore these differences correspond to steady-state incubation dynamics.

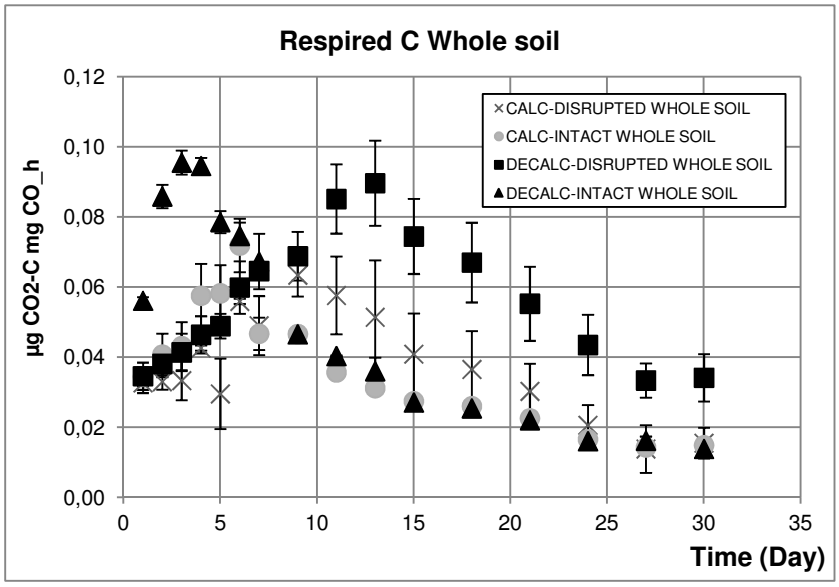

Figure 4. Evolution of C respiration rate (µg CO_2-C mg^{-1} soil OC_h) in 30 days incubation in Calc and Decalc whole soil samples, intact and physically disrupted. Errors bars represent standard errors (n = 3).

Figure 5 shows the evolution of respiration rates in macroaggregates (2-5 mm). In both soils and for disrupted and intact samples, the trend was similar, with an initial increase up to a maximum rate, and a progressive decline over time. However, some differences were observed among soils and in relation to whole-soil samples. No differences were observed for DECALC intact vs. disrupted and CALC intact vs. disrupted macroaggregates at their peak, which occurred almost simultaneously at days 3 to 7. However, in both soils, respiration rates decreased faster and were smaller for intact than for disrupted macroaggregates up to day 21.

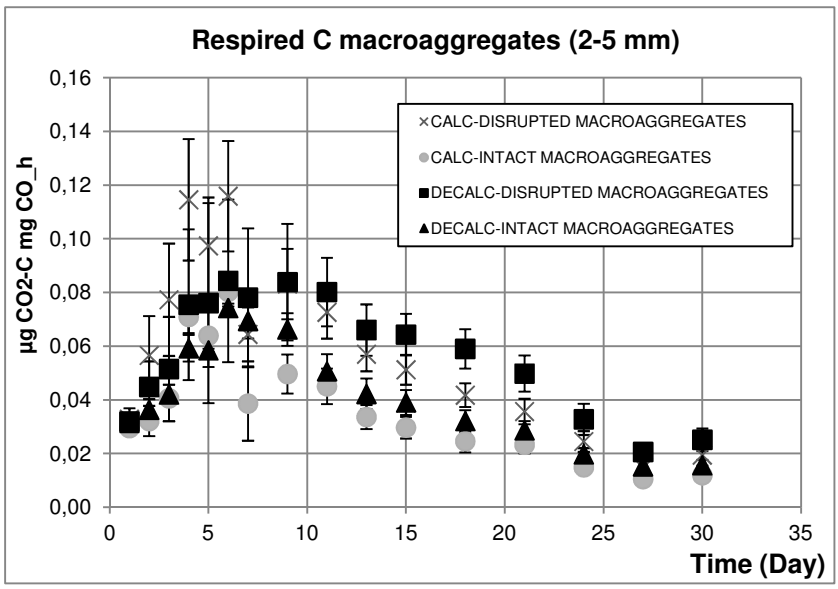

Figure 5. Evolution of C respiration rate (µg CO_2-C mg^{-1} soil OC_h) in 30 days incubation in Calc and Decalc macroaggregates (2-5mm) samples, intact and physically disrupted. Errors bars represent standard errors (n = 3).

DISCUSSION

Despite decades of equal C inputs and soil management, data in Table 1 confirmed previous observations (Fernández-Ugalde et al., 2014) that CALC samples stored more organic C per unit mass than DECALC (Table 1). This result supports the hypothesis of a lower availability for decomposition of the organic matter of the carbonated soil. Soil incubations carried out in this study partially confirmed this hypothesis, as they resulted in a greater total respiration per unit of soil C in DECALC intact than CALC intact soil after 30 days.

Considering the "regulatory gate" theory (Kemmit et al., 2008), abiotic processes such as water and/or gases availability would act as rate-limiting factors for organic matter mineralization rather than microbial processes or organic matter characteristics. Under the light of this theory, differences in porosity and water retention could be at the origin of the observed differences in total mineralization and hourly mineralization rates in our data. Previous work showed that DECALC and CALC soil had a different mineral matrix (Virto et al., 2013). The

differences in fabric were confirmed by our observations of differences in porosity distribution observed among CALC and DECALC soils, both in whole soil samples < 5 mm and in 2-5 mm macroaggregates (Figures 1a and 1b).

However, not much evidence of the role of physical properties in promoting stabilization of organic matter in carbonated soils was found in our study. First, no differences in total porosity that can explain greater mineralization rates in DECALC in the field because of favoured aeration and/or infiltration rates existed. Second, CALC intact samples had the same water holding capacity at field capacity than DECALC intact samples. This means that lower humidity conditions for microorganisms cannot be argued as the reason of lower mineralization rates in CALC when samples are moist at this point.

In relation to physical protection within aggregates, macroaggregates intact samples respiration showed no differences among soils, which suggests that the observed differences in whole-soil samples were not due to organic matter stabilization within macroaggregates. However, the observed greater porosity accessible to microorganisms (0.2-10 μm) in CALC macroaggregates than DECALC macroaggregates indicates that physical conditions were more favourable for microbial respiration in CALC soil. Moreover, the fact that disruption induced greater decomposition in DECALC macroaggregates but not in CALC macroaggregates suggests that organic matter protection was less dependent on the physical protection in CALC macroaggregates, at least at the scale considered in this study.

All in all, the only remaining difference that could explain to some extent the observed differences in mineralization of whole-soil samples would be the greater proportion of pores in the 2.3-6.8 μm pore size class, and lower proportion of pores in the 6.8-10 μm class observed in DECALC in relation to CALC. However, as it has been shown in previous studies (Killham et al., 1993; Strong et al., 2004), organic matter mineralization is slower as the pore size observed decreases, which seems contradictory with the observed greater mineralization in DECALC.

These results suggest that, overall, the greater organic matter protection observed in CALC soil was not related to differences in physical characteristics. Therefore, a different nature of the organic materials stored in each soil or different processes of chemical stabilization in CALC and DECALC soils may be responsible of greater organic matter protection. Respiration rates (Figure 4) suggested indeed that organic matter stored in DECALC whole-soil samples were more labile than those in CALC. This was so for intact and disturbed samples, despite the longer time-lag in respiration peaks in the disturbed samples, probably due to a negative effect of disruption over microorganisms' activity.

As a result, it cannot be excluded that possible differences in the biological lability of organic matter between the two soils could explain the differences in mineralization observed. Biochemical enhanced stability of the organic matter in the presence of Ca^{2+} cations has been already demonstrated (Baldock and Skjemstad, 2000). Moreover, the quality of inherited pedogenic soil organic carbon stored in a soil may determine the resulting amount and quality of the soil organic C remaining after long-term cultivation (Plante et al., 2010). Analyses that confirm differences in the characteristics of soil organic matter when carbonates are present are needed to elucidate to what extent the reaction with the carbonated matrix results in a less biologically labile organic matter.

CONCLUSIONS

Short-term laboratory incubations of intact and physically disrupted whole soil and macroaggregates (2-5 mm) of two neighboring Mediterranean semiarid soils differing in the presence (CALC) or absence (DECALC) of carbonates confirmed previous observations of a greater organic matter stabilization in CALC soil. We attribute this to a reduced bioavailability of the stored organic matter. However, differences in the physical structure of the two soils were insufficient to explain the observed differences in organic matter bioavailability. Therefore, other organic matter stabilization mechanisms are likely responsible of the greater protection of organic matter in the carbonated soil. A greater chemical recalcitrance in

CALC does not seem a likely explanation because, on one hand, the nature and amount of organic inputs was similar for decades in both soils, and on the other hand, recalcitrance *per se* has been shown to be rather rare in soil organic materials (Lutzow et al., 2006). As a result, the reduced biological lability of organic matter observed in CALC could be attributed to a different nature of organic matter in this soil resulting of the interaction between organic materials and the carbonated matrix. This hypothesis needs to be explored, as it has practical consequences both in terms of the amount of organic C potentially stored in these soils, and in the relationship between this C and the soil potential fertility, among other consequences.

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Chapter IV

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ABSTRACT

In the absence of soil inorganic carbon (SIC), total soil carbon can be equated to soil organic carbon (SOC), and dry combustion is considered the most accurate method. In carbonate-containing soils two different methods for measuring SOC have been traditionally used. (1) Wet oxidation and (2) Dry combustion in which SOC is measured either directly by removing inorganic carbon from the samples through an acid pretreatment, or indirectly by subtracting to total carbon the inorganic carbon measured by a calcimeter. But these methods have demonstrated some experimental limitations. An alternative approach is ramped combustion. It takes advantage of the large differential in the temperatures at which SOC and SIC combust or decompose. Our aim in this study was to test the capability of a continuous, ramped combustion technique combining thermogravimetry, differential scanning calorimetry and evolved gas analysis to quantify SIC and SOC in calcareous soil samples, and compare this technique with conventional techniques (wet oxidation, calcimeter, dry combustion). We also sought to use thermal analysis to test the efficacy of acid fumigation for carbonate removal, and to evaluate the effects of acid fumigation on organic matter quality. With that aim a set of 24 soil samples differing in its carbonate content, was used to measure SIC and SOC following these different methods. Results obtained through the three methods were well-correlated, indicating that ramped combustion is an effective method to simultaneously quantify SIC and SOC in carbonated soil samples. Finally, acid fumigation has demonstrated to affect thermal lability of organic matter and incomplete carbonates removal has been observed. Therefore, acid fumigation should be carefully handled when used in SOC quantification and should be avoided for subsequent organic matter quality analysis.

INTRODUCTION

The study of soil organic carbon (SOC) content and turnover is of special importance in agricultural and environmental research. In the last decade the interest in the C cycle has intensified the research efforts in this field. As a result, High-throughput, accurate and inexpensive measurement methods need to be defined (Chatterjee et al., 2009). In the absence of soil inorganic carbon (SIC), total soil carbon can be equated to SOC, and dry combustion is considered the most accurate method (Bisutti et al., 2004), and can be used as a reference to calibrate other methods (Bisutti et al., 2004). Moreover, dry combustion is also the most common method used for measurements of $\delta^{13}C$ in organic carbon turnover studies (e.g. Balesdent and Mariotti, 1996; Denef et al., 2001; Derrien and Amelung, 2011), due to the fact that the coupling of elemental analyzers with IRMS is relatively simple and common. However, in calcareous soils the measurement of soil organic carbon trough dry combustion cannot be direct due to the presence of inorganic carbon. In these soils, the measurement of soil organic carbon remains challenging, and straightforward methods are not yet available (Schmidt et al., 2012).

In carbonate-containing soils (in which SIC accounts for a part of total soil C), two different methods for measuring SOC have been traditionally used. (1) Wet oxidation, which can be considered a “direct” method of measurement, in which the oxidizable fraction (assumed to equal soil organic carbon) is measured leaving intact the inorganic carbon fraction. (2) Dry combustion, in which SOC is measured either directly by removing inorganic carbon from the samples through an acid pretreatment, or indirectly by subtracting to total carbon the inorganic carbon measured by a calcimeter or other method of analysis. Both methods have demonstrated some experimental limitations.

Soil organic C determination by wet oxidation is applied worldwide in many laboratories, particularly in those lacking expensive analytical instruments. It has been the standard method since the early twentieth century, following the protocol described by Walkley and

Black (1934). Soil organic carbon is oxidized while leaving inorganic carbon undecomposed by addition of excess oxidant to air-dried, ground soil samples. Oxidizable carbon is determined indirectly by titration of the remaining oxidant. The main problem of the method is that oxidation of soil organic carbon may be incomplete (Nelson and Sommers, 1996; Skjemstad et al., 1998; Chatterjee et al., 2009). Generally, the oxidized organic carbon represents 75-85 % of the total organic carbon as measured by dry combustion, which makes the use of a correction factor necessary (Grewal and Buchan, 1991). Soil type and the nature of the organic material have been reported as factors influencing the proportion of oxidized carbon (Neal and Younglove, 1993). Therefore the correction factor must be determined experimentally for different soil types. Moreover several additional factors affecting the accuracy of the method, depending on the characteristics or composition of the soil sample, have been reported; Interferences caused by the presence of Cl^-, Fe^{2+}, MnO_2, presence of refractory compounds that may resist oxidation, and uncertainties concerning the redox state of the organic matter, which is assumed to be zero (Bisutti et al., 2004). The interest in using wet oxidation in calcareous soils is the possibility of obtaining a direct measurement of SOC unaffected by the presence of carbonates. However, this method cannot be applied for isotopic studies of SOC.

Soil organic C determination by dry combustion is very commonly used because of the accuracy of the method and the possibility for its use for measurements of $\delta^{13}C$. Its use in calcareous soils is problematic because of the need to differentiate organic versus inorganic carbon. The first approach consists of the subtraction of SIC obtained separately by a calcimeter from the total soil C obtained by dry combustion. This approach has the unavoidable problem of "catastrophic cancellation", which occurs when a relatively small value (organic carbon) is calculated as the difference between two, much larger, measured values (total and inorganic carbon) (Bisutti et al., 2004). This is especially true when the error around each term is greater than the calculated difference. Moreover, this method cannot be used for direct or indirect $\delta^{13}C$ determination of SOC as carbonates are not removed from the soil sample and only the

isotopic composition of total soil C is measured. The second approach, removal of carbonates by acid pretreatment coupled with analysis by dry combustion of samples with and without pretreatment, is the only method applicable for isotopic analysis of organic carbon in soils with carbonates (Midwood and Button, 1998). Most commonly used acids are HCl, H_3PO_4, and H_2SO_3 due to their capability to react with carbonates (Bisutti et al., 2004). Although $\delta^{13}C$ of organic carbon has been reported to remain unaffected after HCl treatment (Midwood and Button, 1998), dissolution in liquid acid and washing may produce organic carbon dissolution and removal during the pretreatment (Midwood and Button, 1998; Bisutti et al., 2004; Chatterjee et al., 2009). Acid fumigation with high concentrated HCl (Harris et al., 2001; Ranmarine et al., 2011) has partially solved the problem, reducing C losses during the treatment. Nevertheless, a correct handling of the method is required and its effects are still not deeply studied.

An alternative approach takes advantage of the large differential in the temperatures at which SOC and SIC combust or decompose. Thermal analysis has been used for decades in soil science but mainly oriented to the study of the soil mineral components, and its application to the study of the organic components has been minor until more recently (Plante et al., 2009a). The combination of various thermal analysis techniques (e.g., thermogravimetry, differential scanning calorimetry) in a single instrument has made them a useful tool in environmental and soil research (Dell'Abate et al., 2003). Subsequent studies have defined the response generated by the oxidation of organic matter in differential scanning calorimetry as the exothermic region between 180-600 ºC (Lopez-Capel et al., 2005; Plante et al., 2005). They have also demonstrated differences in thermal patterns of particular fractions, which suggest the presence of organic matter with different composition, quality or stability. This has made it possible to use thermal analysis techniques in soil organic matter quality studies (Plante et al., 2009b). In relation to the use of this technique for simultaneous analysis of SOC and SIC on soil samples, the potential relies on the fact that SOC results in exothermic reactions at lower temperature and SIC results in endorthermic reaction at higher temeprature. Both result in the

evolution of CO_2, and therefore this changes can be used to derive quantitative estimations of both components.

Our aim in this study was to test the capability of a continuous, ramped combustion technique combining thermogravimetry, differential scanning calorimetry and evolved gas analysis to quantify SIC and SOC in calcareous soil samples, and compare this technique with conventional techniques (e.g., wet oxidation, calcimeter, dry combustion). We also sought to use thermal analysis to test the efficacy of acid fumigation for carbonate removal, and to evaluate the effects of acid fumigation on organic matter quality.

MATERIALS AND METHODS

Table 1. Soil samples set.

Sample ID	Location	$CaCO_3$ (mg g^{-1})	SOC (mg g^{-1})	Clay (mg g^{-1})	Silt (mg g^{-1})	Sand (mg g^{-1})
1	Cuamba, MZ*	0	12.5	196	209	595
2	Pamplona, ES	313	12.0	319	277	404
3	Pamplona, ES	204	12.3	319	277	404
4	Enériz, ES	360	9.1	304	288	412
5	Eneriz, ES	356	10.7	304	288	412
6	Muruzabal, ES	388	7.7	257	353	391
7	Estella, ES	68	8.4	253	215	532
8	Biurrun, ES	5	30.2	193	139	668
9	Biurrun, ES	126	27.8	138	169	693
10	Olite ES	314	10.1	255	571	153
11	Olite, ES	321	14.9	255	571	153
12	Beire, ES	158	14.7	341	233	422
13	Traibuenas, ES	288	7.5	213	328	459
14	Traibuenas, ES	286	9.4	212	362	425
15	Santacara, ES	347	7.2	212	293	495
16	Funes, ES	234	14.1	273	239	488
17	Funes, ES	292	7.9	218	154	647
18	Funes, ES	268	7.5	218	14	612
19	Funes, ES	229	13.3	289	221	489
20	Tudela, ES	337	7.0	381	442	177
21	Tudela, ES	325	13.6	284	445	271
22	Miraflores, ES	156	19.8	18	209	611
23	Miraflores, ES	551	22.1	357	322	321
24	Castañares, ES	0	8.7	116	327	556

MZ: Mozambique; ES: Spain.

A set of 24 surface agricultural soils representing a large range of textures, and presumed carbonate and SOM concentrations were

selected (Table 1). A set of three non-carbonated soils (two from Mediterranean areas and one from SE Africa) were included as controls. These samples correspond to the upper soil profile (0-30 cm on average) of different agricultural and non-cultivated soils from NW Spain, and in the case of non-carbonated soils, one sample from an acid soil in Mozambique. Carbonated samples belong to soils developed on tertiary or quaternary sedimentary materials rich in carbonates. For each soil, disturbed samples were collected with an auger, air-dried and stored in closed containers in the dark for less than three weeks. They were analyzed following three different approaches:

1) Conventional methods: Wet oxidation (Walkley & Black, 1934) and volumetric calcimeter (Allison & Moodie, 1965) for organic and inorganic C determination, respectively.

2) Elemental analyses: dry combustion using a Costech autoanalyzer coupled to a Thermo Finnigan Delta Plus isotope ratio mass spectrometer (IRMS) before and after acid fumigation (AF). Organic carbon was equated to total carbon determined after acid fumigation, and inorganic carbon was estimated as the difference in total carbon before and after acid fumigation. Acid fumigation was performed following the procedure by Harris et al. (2001).

3) Ramped combustion: using a Netzsch STA109 PC Luxx thermal analyzer coupled to a LICOR 840A infrared gas analyzer (IRGA). Quantification of organic carbon was performed using evolved gas analysis of CO_2 (CO_2-EGA) in the exothermic region 120-550℃ associated with organic matter combustion. Inorganic carbon was quantified by CO_2-EGA in the endothermic region 550-850℃ associated with carbonate decomposition. The analyses were conducted in twin samples for each soil, with and without acid fumigation, as for elemental analysis.

Laboratory methods.

Wet oxidation. Oxidizable carbon was determined following the Walkley-Black method as adapated and described in Tiessen and Moir (1993), and used a means of quantifying SOC in each of the soil samples. Concentrated sulphuric acid (10 ml) was added to 1 g soil

sample wetted with a dichromate solution (5 ml). The reaction mixture was shaken and left to stand for 2 hours to allow the oxidation to proceed. Finally, organic carbon (assumed to equal oxidizable C) was determined by back titrating ferrous sulphate with the reactant mixture.

Calcimetry. The carbonate content of each sample was determined using a pressure calcimeter as described in Sherrod et al. (2002) and Fonnesbeck et al. (2013). A mass of 0.5 g of soil was reacted with 4 ml of 6 M HCl in 5 mL of water in a closed reaction vessel. After 2 h, the pressure generated by the CO_2 evolved from carbonates was measured and translated into carbonates concentration in the sample using a calibration curve elaborated with known quantities of $CaCO_3$. Carbonate-carbon was determined by assuming all carbonates were calcium-derived and multiplying the calcium carbonate content by the mass proportion of carbon in calcium carbonate (0.12 g C per g of $CaCO_3$).

Dry combustion. A Costech autoanalyzer coupled to a Thermo Finnigan Delta Plus isotope ratio mass spectrometer (IRMS) was used to determine total C in the soils by dry combustion. Duplicate samples were analyzed. The instrument subjects samples to flash heating at 1350 ºC, and evolved CO2 from combustion was measured using an infrared detector cell. Dry combustion was also carried out following an acid fumigation pre-treatment of replicates of each sample in the set, using the protocol described in Harris et al. (2001). Briefly, 30 mg of sample were moistened to field capacity and subjected to an HCl atmosphere during 24 hours. Fumigation time was calculated to ensure complete carbonate removal of all samples using 0.10 mg C h^{-1} as the rate of inorganic carbon removal, as obtained by Ramnarine et al. (2011).

Thermal analysis methods. Thermal analyses were performed using a Netzsch simultaneous heat flux thermal analyzer (STA 409PC Luxx) equipped with a type-S (Pt/PtRh) TG-DSC sample carrier supporting a PtRh10-Pt thermocouple (Netzsch-Gerätebau GmbH, Selb, Germany). Samples were loaded into an 85-µL Pt/Rh crucible with a lid, with an identical empty crucible used as a reference. Samples were heated from ambient (≈25 ℃) to 105 at 10 ℃ min^{-1}, held at this

temperature for 15 min to remove sample moisture, and then heated to 850 °C at 10 °C min^{-1}. This maximum temperature was found to be sufficient for the sample set as no mass loss or CO_2 evolution was observed. Furnace atmosphere consisted of Ultra-Zero air flowing at 30 mL min^{-1} and N_2 protective gas flowing at 10 mL min^{-1}. Multipoint calibrations for temperature and enthalpy sensitivity were performed using a range of standard inorganic salts (i.e.: RbNO3, KClO4, Ag2SO4, CsCl, and K2CrO4). Evolved gas analysis was performed during the ramped combustion by coupling a LI-840 CO_2/H_2O IRGA (LI-COR Biosciences, Lincoln NE) to the outlet of the STA instrument using Bev-A-Line tubing (0.318 cm inside diameter). A Gelman air filter was used to avoid the entrance of any impurities into the IRGA, and gas flow rates were monitored using an Dakota mass flow meter (Dakota Instruments). CO_2 concentrations of the Ultra-Zero air flowing through the system were baseline corrected to zero using the IRGA software. CO_2-EGA data were converted from partial pressure (ppm CO_2) to carbon mass (µg C), and the cumulative amount of CO_2–C evolved was determined using the path length between the instruments and measured gas flow rates.

An accurate quantification of the organic carbon after acid fumigation was not obtained. The hygroscopic behavior of CaCl produced in the reaction made samples handle very challenging, and losses of sample mass occurred when transferring it to thermal analysis crucible. Consequently, the original sample mass used in the analysis was unknown and organic carbon content could not be referred to an accurate mass sample. Results are still valid for the verification of decarbonation in these samples, as the mere presence of carbonates indicates incomplete decarbonation.

Changes in the composition or structure of the soil organic fraction were assessed qualitatively by observing the differences in the shapes of the exothermic regions of the CO_2-EGA and DSC thermograms with and without acid fumigation.

Statistical analyses. For the whole set of samples (n=24), a comparison of the three approaches used to determine SOC, SIC and

TOC concentrations, was performed. Linear regressions were carried out for SOC with values of the Walkley-Black method as independent and those of dry combustion after fumigation and EGA (120-550 ºC) methods as dependent variables. The Walkley-Black method was chosen as the reference because it is the most widespread method for soil organic C analysis in agricultural soils in many semi-arid regions in which soils contain carbonates. For SIC, linear regressions were carried out with values of the calcimeter method as independent and those of dry combustion (dry combustion-dry combustion after fumigation) and EGA (550-850 ºC) methods as dependent variables. The calcimeter was chosen as the reference method because it is the most frequently used in laboratories running reference agricultural analyses of soil fertility. Finally, linear regressions were also carried out for TC, SOC and SIC for comparing elemental analysis and ramped combustion approaches. In each regression the intercept and the slope values were calculated to evaluate the regression parameters. Intercept of the regression was tested for statistical differences from 0, and the slope was tested for statistical differences from 1. Linear regressions were performed using SPSS 2013, and a statistical significance level of $p \leq 0.05$ was used for all calculations.

RESULTS

Comparison of approaches for inorganic and organic carbon determinations. Table 2 shows the results of SOC and SIC data obtained using the three different approached outlines above.
The correlation among different approaches is shown in Figures 1 and 2.

Table 2. Soil organic carbon (SOC) and soil inorganic carbon (SIC) as measured by the three different approaches used in the study.

Sample ID	Approach 1		Approach 2		Approach 3	
	SOC (mg g^{-1})	SIC (mg g^{-1})	SOC (mg g^{-1})	SIC (mg g^{-1})	SOC (mg g^{-1})	SIC (mg g^{-1})
1	12.50	0.00	18.85	0.42	16.529	0.746
2	12.04	37.56	15.96	28.28	13.668	27.712
3	12.32	24.53	15.59	28.35	12.846	24.948
4	9.10	43.25	8.41	47.61	7.621	43.844
5	10.67	42.77	11.06	46.16	9.731	42.815
6	7.70	46.59	7.23	43.04	5.573	48.280
7	8.43	8.19	9.13	8.06	6.917	7.293
8	30.24	0.58	38.49	0.00	26.064	2.080
9	27.79	15.09	29.58	9.13	27.425	15.133
10	10.07	37.72	9.64	41.09	8.527	38.351
11	14.91	38.48	16.66	39.85	11.507	39.075
12	14.72	18.95	16.17	19.82	12.093	18.918
13	7.47	34.51	9.90	37.85	8.039	36.278
14	9.43	34.34	12.59	37.46	11.149	33.674
15	7.19	41.59	8.81	46.79	5.825	45.550
16	14.05	28.06	14.64	28.84	13.023	28.219
17	7.87	34.99	8.85	37.22	6.723	35.909
18	7.54	32.20	8.62	35.74	7.052	33.993
19	13.32	27.54	14.13	29.29	10.633	28.738
20	6.96	40.47	8.37	40.03	5.704	40.429
21	13.61	38.98	14.40	34.90		
22	NA	18.67	NA	24.00	7.521	23.581
23	NA	66.11	NA	61.48	6.843	70.289
24	8.70	0.00	9.47	0.00	6.813	0.421

The inorganic carbon concentration of the samples obtained with calcimeter were compared to the results obtained by elemental analysis (EA) in the second approach, and evolved gas analysis (EGA) in the third approach (Figure 1). High R^2 values were obtained for both regressions, though the R^2 value obtained by EGA (0.975) was greater than R^2 value obtained by EA (0.949). In both analyses, the slopes of the regressions (b_{EGA} = 1.012 ± 0.036 and b_{EA} = 0.993 ± 0.049) were not statistically different from 1, and the intercepts in

both cases (a_{EGA} = 0.266 ± 1.192 and a_{EA} = 0.755 ± 1.65) were also not statistically different from 0.

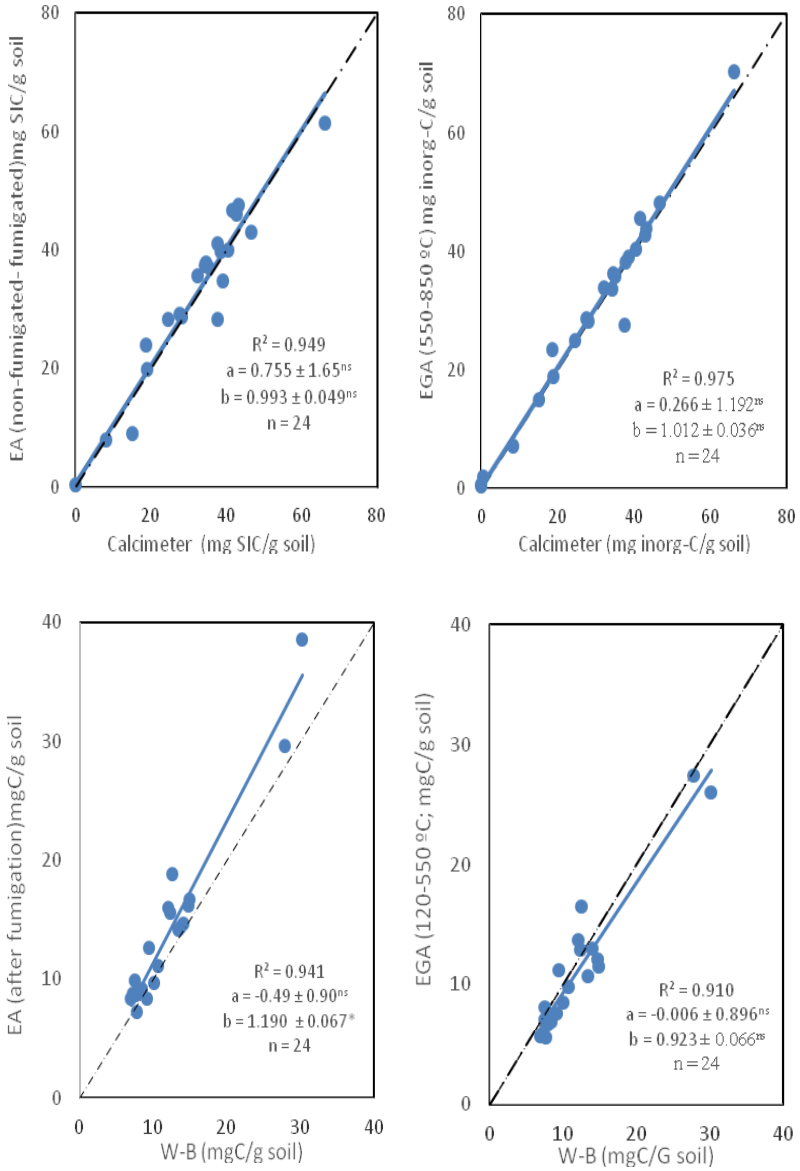

Fig. 1 Linear regressions of SIC and SOC concentrations as measured by elemental analysis (EA) and evolved gas analysis (EGA) compared with SIC measured by a calcimeter and SOC measured by Walkley-black wet oxidation method (W-B). An asterisk after the coefficients (a, intercept, or b, slope) indicates that it is significantly different (*t* test, p≤0.05) from 0 (a, intercept) or 1 (b, slope); ns denotes no significant difference.

The organic carbon concentrations of the samples obtained by WalkleyBlack in the conventional approach were compared to the results obtained with elemental analysis (EA) in the second approach, and evolved gas analysis (EGA) in the third approach. Both EA and EGA results showed a strong correlation with Walkley-Black (Figure 1). The R^2 value obtained by EA (0.941) was greater than the R^2 value obtained by EGA (0.910). In the regression with EGA, the slope (b = 0.923 ± 0.066) was not statistically different from 1 and the intercept (a = -0.006 ± 0.896) was not statistically different from 0. In contrast, the slope obtained in the regression with EA (b = 1.190 ± 0.067) was statistically different from 1, being the values obtained by EA higher than by WB in the samples with the highest concentrations. The intercept (a = -0.489 ± 0.899) was not statistically different from 0.

The results obtained through elemental analysis (EA) and evolved gas analysis (EGA) were compared (Figure 2). High correlations between both methods were found for total carbon (R^2 = 0.947), inorganic carbon (R^2 = 0.965) and organic carbon (R^2 = 0.975) measurements (figure 2). In the regression analyses for total and inorganic carbon, no significant differences were observed in slope or intercept. However in the case of organic carbon the coefficients, slope (b = 0.796 ± 0.028) and intercept (a = -2.316 ± 0.434) were significantly different from 1 and 0 respectively. Consequently, a bias exists among both method and the differences among the values measured with both methods increase with increasing values (Figure 2).

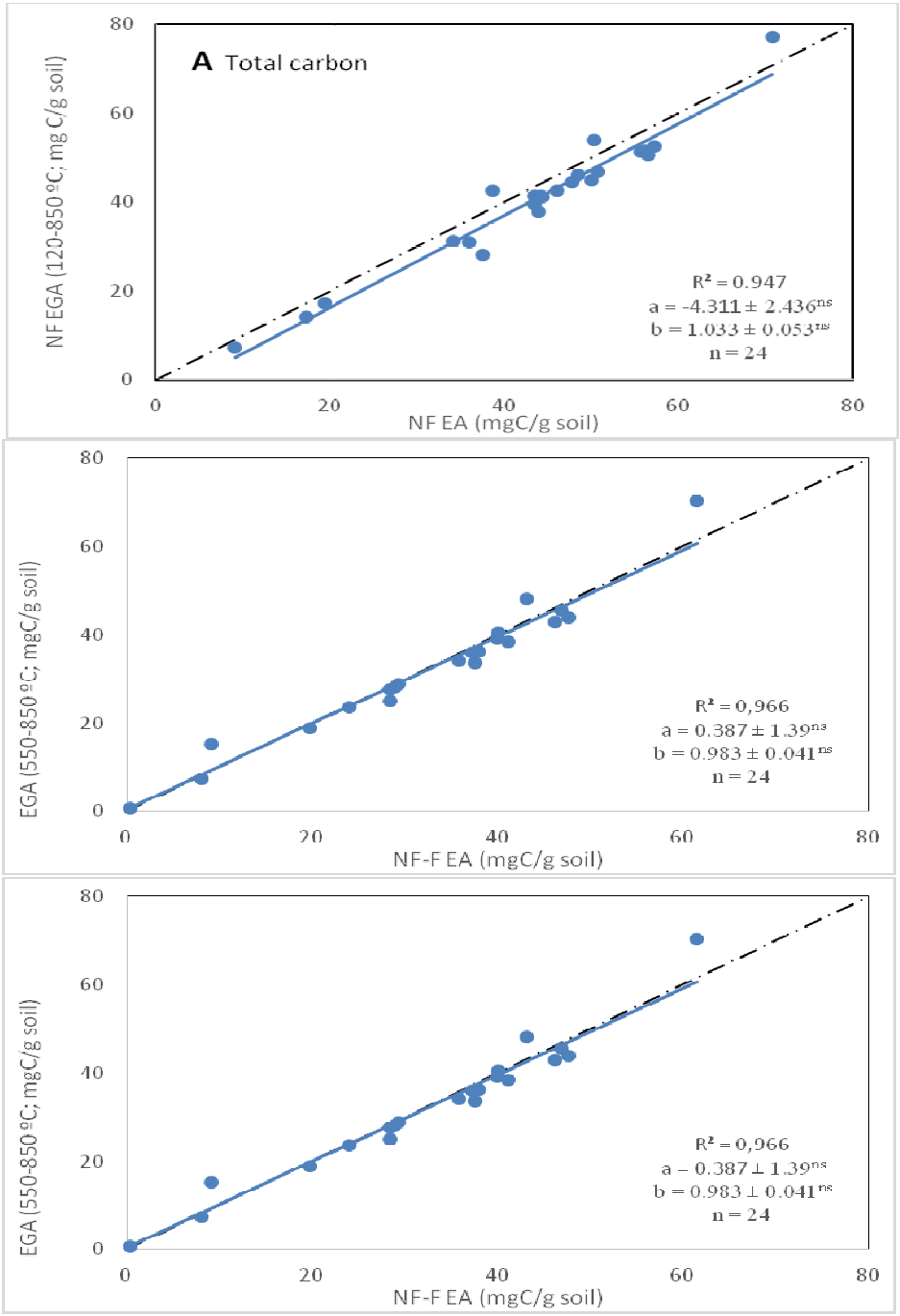

Figure 2. Comparison by linear regressions of total, soil organic and soil inorganic carbon concentrations as measured by elemental analysis (EA) and evolved gas analysis (EGA) methods. An asterisk after the coefficients (a, b) indicates that it is significantly different (*t* test, p≤0.05) from 0 (intercept) or 1 (slope); ns denotes no significant difference.

Acid fumigation.

Efficacy of acid fumigation for decarbonation. Ramped combustion of untreated and acid fumigated samples made it possible to determine if samples were completely decarbonated by the acid fumigation treatment. Qualitative evidence of incomplete decarbonation can be observed in both the endothermic region of the DSC thermogram near 700 ºC (data not shown), and the associated peak in the CO_2-EGA thermogram (Figure 3a). The large peaks present in untreated samples are dramatically reduced by acid fumigation in carbonate-containing samples, but a small peak is still typically present in the acid fumigated samples. In table 3, it can be observed that a certain amount of inorganic carbon was still present in the samples after acid fumigation.

Table 3. Inorganic carbon (mg C g^{-1} soil) measured by evolved gas analysis (EGA) in fumigated and non-fumigated samples.

Sample Nº	Non Fumigated	Fumigated	Non removed IC (%)
1	0.75	0.21	-
2	27.7	1.86	6.71
3	24.9	2.16	8.67
4	43.8	-	-
5	42.2	2.04	4.76
6	48.3	0.87	1.79
7	7.29	0.72	9.88
8	2.08	-	-
9	15.1	4.53	29.9
10	38.3	0.98	2.56
11	39.1	1.71	4.38
12	18.9	1.63	8.63
13	36.3	0.73	2.02
14	33.7	1.16	3.43
15	45.5	0.85	1.86
16	28.2	2.14	7.58
17	35.9	0.75	2.09
18	34.0	1.02	3.00
19	28.7	1.97	6.86
20	40.4	0.57	1.40
21	-	1.59	-
22	23.6	1.65	7.00
23	70.3	0.93	1.32
24	0.42	0.29	-

Assuming that the quantitative estimations are valid, this portion of remaining inorganic carbon varied between 1 and 10 % of the original inorganic carbon in most samples, except in sample 9, where it was of almost 30%, despite this sample not having the highest initial SIC content. In any case, this observation shows that the proportion of remaining SIC was not related to the original concentration (see SIC concentration data in the non-fumigated column in Table 3 and in Table 2).

Effects of acid fumigation on soil organic matter. Qualitative observations of the CO_2-EGA thermograms during ramped combustion showed substantial differences in the thermal behavior of organic carbon between untreated and acid fumigated samples (see example for samples 9 and 24 in Figure 3). The shape of the exothermic region (150-550 ºC) of the curves in both samples change after acid fumigation, being the evolved CO_2 smaller at low temperatures in the fumigated sample. Moreover in sample 9 the evolved CO_2 at high temperatures is higher after fumigation.

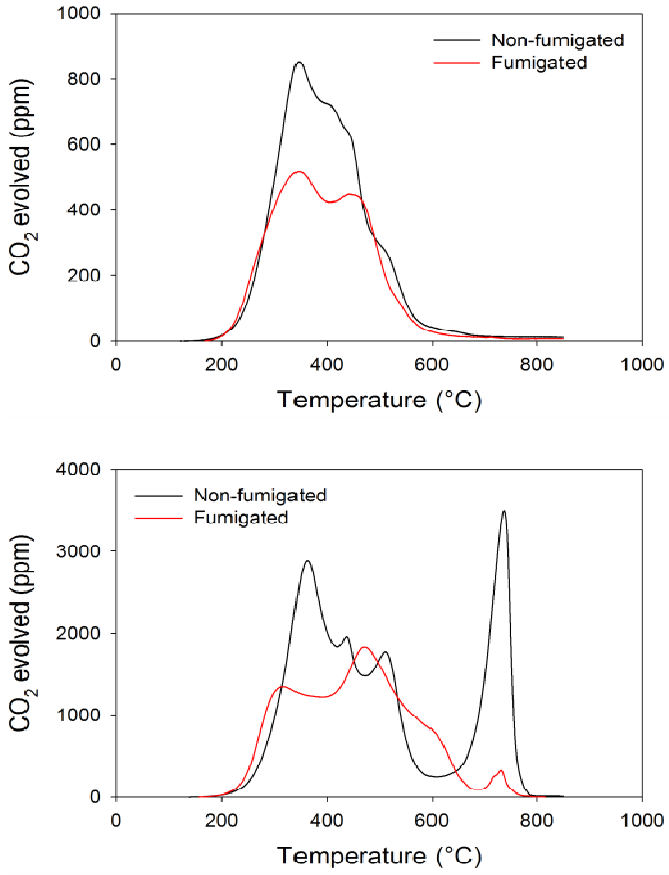

Figure 3. *Evolved gas analysis - CO_2.* Curves generated in the analysis of samples 24 (top) and 9 (bottom) before AF (non fumigated) and after AF (fumigated). Peaks in the exothermic region (120-550 ºC) correspond to CO_2 evolved in OC combustion. Peaks in the endothermic region (550-850 ºC) correspond to CO_2 evolved in IC combustion.

DISCUSSION

A ramped combustion approach using simultaneous TG-DSC-EGA presents some advantages and disadvantages for measuring C concentrations in carbonated soils. First, this method allows the measurement of total, organic, and inorganic carbon from a single sample, which ensures a higher methodological precision by removing

the problem of error propagation when different subsamples of one sample are used for different analysis. A second advantage is in avoiding acid fumigation for carbonate removal, and the possible associated problems, such as incomplete decarbonation, changes in organic matter composition and possible losses of labile organic carbon. The problem of "catastrophic cancellation" associated to using different analysis and subtracting C concentrations from different magnitude orders can be avoided using this technique. Finally, thermal analysis can be used a method to verify carbonate removal effectiveness and the degree of alteration of SOM associated to fumigation. Although the use of acid fumigation is not always required for studies interested in isotopic analysis of the organic carbon as some advances have been done in coupling TA with IRMS (Manning et al., 2008), reality is that most studies of this type are done removing carbonates from soil samples using acid fumigation. They seem to have the only instrument in operation, but it is technically possible.

However, this method has also some disadvantages. In general, the method is very slow compared to EA, but might save time in the end since there is no sample preparation (acid fumigation) and it needs only run one sample for a TOC, SOC and SIC analyses. Second, the use of a single cut-off temperature for SOC vs SIC could be problematic depending on the nature of the carbonate present, which decomposes at different temperatures. This could be avoided by using sample-by-sample approach and/or peak deconvolution for a more precise quantitative approach. Finally, the instrumentation is not common yet, though at present it is comparable in price to an EA and has very low consumables costs.

Comparisons with conventional methods demonstrated that ramped combustion using a thermal analyzer coupled to an infrared gas analyzer is an effective method to quantify organic and inorganic carbon in carbonated soil samples using one sample per analysis. Moreover, the results obtained in the quantification of both types of carbon through analysis of the evolved CO_2 have shown high correlations with conventional methods of carbon determination (Figures 1, 2). Despite of the high correlations among methods, significant differences appeared in the slope of OC measurement

methods regressions, when dry combustion results were compared with wet combustion and thermal analysis results (Figure 1 bottom left). This seems especially true for samples with high SOC contents. These differences might be due to incomplete decarbonation of the samples after acid fumigation (Table 2), or to incomplete combustion.

In a recent study Vuong et al. (2013) also observed high correlation among the results obtained for SIC and SOC with thermal gradient method and dry combustion in calcareous soils. In that case wet oxidation was not included in the study. Our results show also high correlations with wet oxidation, which is interesting due to the fact that this method is applied in many laboratories worldwide, and many existing soil data sets have been built with values obtained through this method.

The results of thermal analysis after fumigation of the samples with HCl have shown its effectiveness in the removal of most of the carbonates present in soil samples, but complete decarbonation was not obtained in any of the samples with no relation to the original carbonates content. In previous studies, Harris et al. (2001) and Ranmarine et al. (2011) claimed complete decarbonation of the samples through the method of acid fumigation. This was affirmed after observing that $\delta^{13}C$ of the sample remained constant while acid treatment period were increased, which is not necessarily a proof of edivence of the organic fraction not being affected. Uncertainties about the completeness of inorganic removal have been highlighted as a problem associated to acid treatments (Bisutti et al., 2004). Midoow and Boutton (1998) found that long periods of time (up to three days) could be necessary for complete decarbonation of soil samples with HCl. Others have pointed out several mechanisms that may cause incomplete decomposition of carbonates. Acid treatments with H_2SO_3 produce calcium sulfite which may form coatings that prevent carbonates decomposition (Fernandes and Krull, 2008). Incomplete removal of small quantities of acid-resistant carbonates (e.g. dolomite, siderite) present in soil in a low proportion (Schmidt et al., 2012) could be responsible of remaining carbonates. Finally, some authors have reported organic matter protection of some carbonates from acid attack in shelly calcarosols (Schmidt et al., 2012).

Therefore, this technique must be handled carefully in studies for determining carbon stocks or carbon sequestration and complete decarbonation should be confirmed when acid fumigation is used, in order to avoid overestimation of SOC. Special care must be taken in studies of the organic matter cycle using isotopic techniques, due to the great effect of the presence of carbonates in the C isotopic signature of soil samples.

Another concern about the use of acid pretreatments in soil samples is the possible effects on soil organic matter. This is an important issue due to the possible implications in subsequent analyses on the nature of organic matter. We expected that the comparison of results of thermal analysis of soil samples fumigated and non-fumigated could produce interesting information about the effects of acid fumigation. Qualitative changes in thermal lability were observed (Figure 3) trough the curves obtained with EGA. The shape of the curves indicates a possible loss of the most labile organic matter, or a transformation to more thermal resistant compounds.This is in accordance with Caria et al. (2011) who concluded that hydrochloric acid in contact with soil alters the thermal properties of organic materials and makes them less volatile. Moreover, some EGA results curves (Figure 3) indicate losses of total organic carbon. Contrary to Harris et al. (2001), who found that no losses of organic carbon occurred when treating non carbonated samples with this acid fumigation, several authors have reported losses of organic carbon after acid treatments (Bisutti et al., 2004; Fernandes and krull, 2008). In accordance with our results, further evidence is needed to ensure that no organic carbon losses occur after HCl fumigation of carbonated soil samples.

CONCLUSIONS

The use of a ramped combustion, thermal analysis technique has proven capable of quantifying inorganic and organic carbon fractions in carbonated soil samples. This method has also obtained precise and well-correlated measurements with the most commonly used conventional methods (e.g., wet oxidation and elemental analysis).

Thermal analysis also avoids the use of acid treatments necessaries when using elemental analysis. Acid fumigation with HCl has been also tested in the present study. Even though acid fumigation has shown to be highly effective for carbonates removal, it may be incomplete. Therefore it should be handled carefully, and special care must be taken when used in isotopic studies of the organic fraction of soils. Moreover, acid fumigation has proved to affect organic matter thermal lability, so subsequent organic matter quality analysis will be influenced.

The potential of this technique for studies of C in soil samples from carbonated soils is therefore high.

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Chapter V

Irrigation Induced Changes in Soil Quality Indicators within a Semi-arid Mediterranean Soil

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ABSTRACT

A new irrigation canal in Navarre, northeastern Spain, has increased the irrigated area by 22,000 ha in the past five years, and the overall amount of irrigated land in the Mediterranean region is expected to triple in the next few decades. Our objective was to determine if this management change would change the importance and sensitivity of soil quality indicators identified previously for non-irrigated crop management at this site. Soil physical, chemical and biological properties were measured again after three years of irrigation management within conventional tillage, minimum tillage and no-tillage treatments. Principal component analysis (PCA) was applied to identify the most sensitive indicators for 0-5 and 5-15 depth increments, and SMAF (Soil Management Assessment Framework) was applied for soil quality (SQ) evaluation. A change of the main SQ indicators after just three years was observed. Such a fast change ratifies the precocity of some of the previously selected indicators (e.g. POM). And confirm the necessity of reevaluating SQ indicators when agroecosystem conditions are modified. The results obtained by the use of SMAF revealed that this framework resulted sensitive to the observed differences in SQ among soil tillage managements, and were therefore adequate to evaluate SQ differences among them. Even though, a detailed analysis of some of the interpretation algorithms seems necessary to adapt the SMAF to the local agroclimatic conditions. This seems especially important in those parameters for which systematic low values where obtained (e.g. BD).

INTRODUCTION

Soil is a vitally important natural resource because of the many environmental and life-sustaining processes it supports. To help ensure this vital resource is not being degraded by inappropriate soil and crop management decisions, various approaches and frameworks for soil quality (SQ) evaluation have been proposed (e.g. Andrews et al., 2002; Sharma et al., 2008; Aziz et al., 2013; Karlen et al., 2013; Yao et al., 2013). Being aware of soil physical, chemical, and biological conditions is a basic step for soil conservation, especially in intensely managed agroecosystems.

The primary challenge associated with SQ assessment is that in contrast with water and air quality, SQ cannot be directly evaluated. For instance, with regard to human welfare, the functions soil must provide (*i.e.*, provision of food, feed, fiber, fuel; a construction medium; sanctuary for biological diversity) are very diverse (Carter et al., 1997). Consequently, the concept of SQ has been somewhat controversial among soil scientists and consensus has been difficult to build (Karlen et al., 2003). A first step toward consensus was achieved in 1997, when a Soil Science Society of America committee characterized SQ as "the capacity of a specific kind of soil to function, within natural or managed ecosystem boundaries, to sustain plant and animal productivity, maintain or enhance water and air quality, and support human health and habitation" (Karlen et al., 1997). This definition supported the multifunctional concept of soil, in contrast to previous visions focused solely on crop production (Singer & Erwin, 2000). Together with this definition and based on previous ideas from Doran & Parkin (1994), five critical soil functions were proposed. These are: (1) sustaining biological activity, diversity, and productivity; (2) regulating and partitioning water and solute flow; (3) filtering, buffering, degrading, immobilizing, and detoxifying organic and inorganic materials, including industrial and municipal by-products and atmospheric deposition; (4) storing and cycling nutrients and other elements within the Earth's biosphere; and (5) providing support of socioeconomic structures and protection for archaeological treasures associated with human habitation.

To quantitatively evaluate how well a specific soil is performing these functions, SQ indicators must be selected and assessment tools must be developed. The use of indicators to monitor SQ was first applied through the use of pedotransfer functions (Larson & Pierce, 1991). Doran et al. (1996) later described those indicators as soil properties and processes with the greatest sensitivity to changes in the critical soil functions. According to these authors, indicators should correlate well with ecosystem processes, integrate soil properties and processes, be accessible to many users, be sensitive to management and climate changes, and, when possible, be components of existing databases. Unfortunately, selection of a minimum data set (MDS) of SQ indicators cannot be universal because of the site-specific nature of soils and multitude of different functions they must perform. Therefore, MDS selection for SQ assessment must be determined by which functions are most critical to meet management goals (Harris et al., 1996; Andrews et al., 2004). Furthermore, because of the site-specific nature of SQ, different soil property measurements may be required for a specific function (e.g. productivity) depending upon the specific agroecosystem for which the assessment is being made (Govaerts et al., 2006; Rezaei et al., 2006; Shukla et al., 2006; Yemefack et al., 2006; Imaz et al., 2010). Selection of proper SQ indicators must therefore be carefully accomplished for different situations, taking into account changes in land-use, soil management, or hydrologic conditions that may affect SQ.

To address the complexity of SQ evaluation, the use of soil management assessment tools and integrative indexes that included biological, chemical and physical soil data, was an interesting advance for assessing sustainability of soil ecosystem management practices (Andrews & Carroll, 2001). As a result, various soil management assessment tools have been proposed. The Soil Management Assessment Framework (SMAF) proposed by Andrews et al. (2004) has been tested in a wide variety of locations throughout the U.S., including Georgia, California, and the Pacific Northwest (Andrews et al. 2002; Andrews et al. 2004); Colorado (Zobeck et al., 2008); Iowa and Wisconsin (Karlen et al. 2006; Sttot et al., 2011; Karlen et al., 2013); and the Great Plains (Wienhold et al. 2006; Liebig et al., 2006). When compared with other assessment tools, the SMAF

has demonstrated a high sensitivity for differentiating among contrasting soil management practices (Zobeck et al., 2008). The SMAF is based on quantifying effects of soil and crop management on dynamic soil properties and overall soil functioning. It is designed to follow a three step system, namely (1) indicator selection, (2) indicator interpretation, and (3) integration into a SQ index value. In the first step, SMAF serves as an expert system to help select appropriate SQ indicators for the selected management goals, associated soil functions, and site-specific factors including region, crop climate, sampling time and analytical methods (Wienhold et al., 2009). Non-linear algorithms or scoring curves for up to 14 indicators are currently incorporated in the SMAF. The scoring curves convert measured values for the selected indicators into relative values ranging from 0 to 1. A relative score of 1 means that indicator is non-limiting with regard to the specific soil function being evaluated (Andrews et al., 2004). After scoring each indicator, the relative values of the individual indicators can be combined into an overall index, that can be used quantitative comparisons of soil management strategies on the same soil resources and for temporal comparisons within the same field, watershed, or other sampling location.

Semi-arid Mediterranean agroecosystems have been shown to have different degrees of SQ, mostly due to human pressure and climate factors (e.g. Sánchez-Navarro et al., 2015). Previous intensive cereal production studies on dryland soils in this region have shown that SQ indicators related to the soil organic fraction and physical condition are especially suitable for evaluation of these situations (Imaz et al., 2010), and that changes in management can induce significant improvements in SQ (Fernández-Ugalde et al., 2009). An emerging change for these production systems is the introduction of irrigation which implies a major change in “soil climate” and potentially in the organic C cycle (Apesteguía et al., 2015). To date, the consequences of imposing irrigation on SQ in this area have not been evaluated. The current study was conducted in a semiarid area where irrigation was recently introduced. Our hypothesis was that SQ indicators selected for the area in previous non-irrigated studies (Bescansa et al., 2006; Virto et al., 2007; Imaz et al., 2010) would still be the most sensitive. We followed a step-wise protocol to: (1) identify

the most sensitive SQ indicators for evaluating long-term tillage and residue management strategies within an irrigated Mediterranean agroecosystem, (2) compare the indicators selected under irrigated conditions with those previously selected for dryland conditions, (3) evaluate the overall SQ for the irrigated tillage treatments using the SMAF, and (4) determine if the SMAF was appropriate for SQ analysis under irrigated Mediterranean conditions.

MATERIALS AND METHODS

Experimental site. The experimental site was located in Olite (Navarre, NE Spain) (42°27'19''N; 18°10'00''W; 402 m a.s.l.), and had been used for cereal production for decades. It was traditionally managed using conventional tillage (moldboard plow), until in 1994, it was converted into an experimental plot including different crops and tillage treatments for cereal production under dryland conditions. In response to the development of a regional irrigation plan in 2009, which included the transformation of 55,000 ha from non-irrigated to irrigated production, the experimental site became irrigated, but the long-term tillage treatments were maintained. The soil in this site is a fine-clayey Calcic Haploxerept (Soil Survey Staff, 2006). Climate is described as dry sub-humid (C1B'2db'4 according to the classification of Thornthwaite (1948)). Mean annual evapotranspiration is 740 mm and mean monthly temperature is 13.5 ºC. The average annual precipitation is 525 mm 18% of which falls during the summer (July-September).

Experimental design. The experimental design was a randomized block with four replications. Plots were 9m x 24m in size. Barley (*Hordeum vulgare* L.) was planted each year at a sowing rate of 160 kg ha^{-1}. A total of six soil and crop residue management practices were included in each block, three of which were evaluated for this study. The three practices represented different tillage intensities: conventional tillage (CT), minimum tillage (MT), and no-tillage (NT). Conventional tillage consisted of mouldboard ploughing (0.25 m deep) in late summer, followed by secondary tillage with a

harrow for seedbed preparation before seeding (late October). Crop residues were incorporated into the arable layer during tillage. Seeding was accomplished using a coulter-seeder. Minimum tillage consisted of chisel ploughing (0.15 m deep) and secondary tillage and seeding as for CT. A direct seeder that opened a seed-furrow 30–50 mm deep was used for NT. Nitrogen and P fertilization was similar for all treatments, averaging 100–27–0 kg N–P–K ha^{-1} $year^{-1}$. Superphosphate was used as basal dressing in September every other year. Urea was used every year for N fertilization.

Soil and crop sampling. For this study, soil samples were collected 3 years after the conversion to irrigation. Disturbed and undisturbed samples were collected for the various analyses. Disturbed soil samples were collected for the 0–5- and 5–15-cm depth using an Edelman type auger (Ø = 5 cm). Five subsamples were collected per plot for each depth increment and combined to obtain a composite sample for chemical and physical analyses. Immediately after sampling, a portion of the composite soil sample was gently pushed through an 8 mm sieve. These aggregates were allowed to air dry and used to for aggregate stability determinations (see below). The remainder of the soil was air-dried and ground to pass a 2 mm sieve. Undisturbed core samples were collected in triplicate using bevel-edged steel rings (Ø = 5 cm, total volume = 100 cm3) for the 0–5- and 5–15-cm depth increments to determine soil bulk density (BD) and for the 0–15-cm depth to determine soil water retention characteristics. A second samples collection was accomplished in order to obtain soil samples needed for earthworms and microbial biomass measurements in spring. Two 20x20x20 cm soil blocks were extracted in each plot where earthworm activity was observed. Then earthworms of each block were manually selected and kept for density analyses (g earthworms biomass m^{-2}). Unaltered soil samples were also collected for the 0–5- and 5–15-cm depth increments and kept refrigerated for microbial biomass analysis.

Throughout the period of record (2010-2012), barley was harvested each year with plot-scale combines and grain yields were recorded. Grain weights were taken directly on the combine and grain samples from each plot were collected and analyzed to determine

water content. Grain yields were then adjusted to a constant water content of 120 g kg^{-1}.

Soil analyses. Selected soil physical, chemical and biological properties were measured as potential SQ indicators. Pre-selection of these indicators was based soil parameters previously detected to represent soil functions and found to be sensitive to the management practices under dryland conditions in the same experimental field (Imaz et al., 2010).

Physical properties. Bulk density (BD), penetration resistance (PR) and soil aggregate stability (MWD_d, MWD_w and MWD_w/MWD_d) were measured as the physical soil parameters. Available water-holding capacity of the soil (AWHC) was also measured and included in SMAF analysis as a well-known SQ indicator in the region (Bescansa et al., 2006).

The core method was used to determine bulk density. Penetration resistance was measured 6 months after seeding at 9 points per field replicate to a depth of 60 cm using a field penetrometer (Rimik CP20, Agridy Rimik Pty Ltd, Toowoomba, Qld, Australia). Measurements were made after a rainy period to avoid differences in moisture content among treatments. Measurements were recorded every 15 mm, and PR for 0 to 5 and 5 to 15 cm were calculated as weighted depth averages. Dry aggregate stability was determined by placing 100 g of dry aggregates (<8 mm) in the top of a column of sieves of 6.3, 4, 2, 1, 0.5 and 0.25 mm openings and shaking the whole in a rotary movement at 60 strokes/min for 60 s in a Retsch VS 100 device (Retsch GmbH & Co., Haan, Germany). For wet aggregate stability, a constant shower-like flux (6 L/min) of distilled water was applied from the top of the same set of sieves while sieving (60 strokes/min, 60 s). Equal initial aggregate distribution for wet and dry sieving for each sample was ensured by using a mechanical sample divisor (Retsch GmbH & Co., Haan, Germany). Aggregate size distribution was expressed as the mean weight diameter (MWD) after dry and wet sieving. Stability of the aggregates was evaluated using a ratio of wet-to-dry MWD (MWDw/MWDd), as proposed by Lehmann et al. (2001) and Franzluebbers (2002). Soil water retention (SWR) at -33 kPa was determined using undisturbed soil samples, while at -1500 kPa,sieved

(<2 mm) soil samples were placed on pressure plate extractors (Soil Moisture Equipment Corp., Santa Barbara, CA). Volumetric SWR values were calculated using BD. AWHC was calculated from the difference in soil volumetric moisture content at field capacity (-33 kPa) and wilting point (-1500 kPa) in pressure plate extractors (Soil Moisture Equipment Corp., Santa Barbara, CA), as described by Dirksen (1999).

Chemical properties. Total N was determined using the Kjeldahl digestion procedure. Available P was determined as described by Olsen and Sommers (1982). Exchangeable K was quantified using atomic absorbance after extraction with NH4OAc 1N (Knudsen et al., 1982). Soil electrical conductivity (EC) and soil pH were measured in distilled water (1:2.5). Soil pH was determined with a Crison GLP22 pHmeter (Crison Instruments, S.A., Barcelona, Spain). Conductivity was read with a Crison GLP32 conductivity meter (Crison Instruments, S.A., Barcelona, Spain). Carbonates content was measured in a Bernard's calcimeter by quantifying the CO2 produced after treating a soil sample (<2 mm) with HCl.

Organic matter and soil biological properties. Soil organic C (SOC) was determined by wet oxidation (Walkley-Black). The fraction of organic matter corresponding to particulate organic matter >53 mm in size (POM, Cambardella and Elliot, 1992) was isolated by dispersion and sieving of 10 g of air dried soil, using the method described in Virto et al. (2007). Two soil blocks (20 cm x 20 cm x 20 cm) were taken in each plot in May (spring, physiological maturity of barley) for earthworm activity determination. Earthworms were sampled by hand-sorting and counted in the field. Individuals were weighed (fresh weight basis) in the laboratory, fixed with ethanol-formalin, and preserved in 10% formalin (Baker and Lee, 1993). Microbial biomass carbon (MBC) was measured by comparing non fumigated and chloroform ($CHCl_3$) fumigated 15 g soil samples. Carbon determination was done by sulfuric digestion in the presence of chromic acid as extractant dissolution and subsequent spectrophotometry.

Soil Quality indicators (SQI) selection. The selection of the most adequate SQ indicators to determine the MDS for this particular soil and soil use (*i.e.*, irrigated crop production) was done as described by Imaz et al. (2010) for dryland conditions. First, univariate analysis of variance (ANOVA) for the different soil variables to examine significant influences in management and depth were run. Only those variables for which the F statistics for soil management was significant ($P < 0.05$) were retained for further analysis. Factor analysis was then used to group the retained variables into statistical factors based on their correlation structure. Principal component analysis (PCA) was used as the method of factor extraction (Brejda et al., 2000). To eliminate the effect of different units of variables, factor analysis was done using the correlation matrix on the standardized values of the measured soil properties, so that each variable had mean = zero and variance = 1 (total variance = number of variables; Shukla et al., 2006). We used the determinant of the correlation matrix as an indicator to identify the existence of correlations among variables.

Using the correlation matrix, principal components (factors) with eigenvalues >1 were retained and subjected to varimax rotation with Kaiser to estimate the proportion of the variance of each attribute explained by each selected factor (loadings), and by all factors (communalities). A high communality for a soil attribute indicates that a high proportion of its variance is explained by the factors. In contrast, a low communality for a soil attribute indicates much of that attribute's variance remains unexplained. Less importance should be ascribed to soil attributes with low communalities when interpreting the factors (Brejda et al., 2000). To evaluate the effects of the studied tillage and residue management treatments on the extracted factors, factor scores for each sample point were calculated and ANOVA was performed on the new score variables. Homogeneous groups among treatments were detected using Duncan's test ($P < 0.05$, unless otherwise indicated). Only factors that differed among treatments were retained for further consideration. Soil attributes were then assigned to the factor for which their loading was the highest (Shukla et al., 2006). For each retained factor, highly weighted attributes were

selected as possible SQI. We considered highly weighted as those within 10% of the highest factor loading, as in Andrews et al. (2002b) and Rezaei et al. (2006). When more than one soil attribute was included within this range, they were subjected to redundancy analysis to determine whether all or any of them were correlated, and thus could be eliminated from our list of selected SQI.

Soil quality evaluation. The soil management assessment framework (SMAF) developed by Andrews et al. (2001) was used for SQ evaluation. In a first step, several soil and ecosystem parameters (organic matter class, texture class, clay content, mineral class, Fe_2O_3 class, climate class, season code, region code, crop code, rotation code, slope class, weathering class, P method and EC method) were introduced in order to adapt SAMF interpretation algorithms to our local conditions. Then, the values obtained for several physical, chemical and biological soil parameters included in SMAF and previously measured for the soil indicators selection process (BD, PR, AWHC, aggregates stability, P, K, pH, EC, TOC and MBC) were introduced in the assessment tool. Univariate analysis of variance (ANOVA) was run to examine significant differences among the scores given by SMAF for the different soil parameter and the general SQ index generated.

RESULTS

Crop yields. Average yields were not significantly different among managements and were within the range of average local yields during the studied years (Table 1).

Table 1. Effect of tillage system on barley yield

Average (2010-2012)	**Tillage system**		
	NT	MT	CT
Yield (Mg ha^{-1})	**4.10 a**	**4.38 a**	**4.47 a**

Values within the same row followed by a different uppercase letter are significantly different among treatments at $P<0.05$.

Identification of soil quality indicators. ANOVA showed that some of the studied soil properties were affected by soil management. At the first depth (0-5 cm), seven of the measured parameters were significantly different; BD and PR among physical properties, N and K

among chemical properties, and TOC, MBC and earthworm among organic matter and biological properties (Table 2). In the second depth (5-15 cm) four of the measured parameters were significantly different; BD and PR among physical properties, N among chemical properties, and TOC among organic matter and biological properties (Table 3). Only those parameters, which were observed to be sensitive to management, were taken into account in further analysis for obtaining the most suitable SQ indicators. POM-C was also included in further analysis as it was the most sensitive indicator in dryland conditions (Imaz et al., 2010) and it was significantly different among treatments at $P<0.1$ in 0-5 cm depth increment (Table 2).

Table 2. Results of the analysis of variance (ANOVA) in the 0-5 cm depth for physical, chemical, organic matter and biological soil properties.

Soil properties	No-tillage	Minimum tillage	Conventional tillage
Physical			
BD ($Mg.m^{-3}$)	1.64^{a}	1.51^{b}	1.53^{b}
PR (Mpa)	0.87^{a}	0.41^{b}	0.5^{b}
MWD_d(mm)	4.01	3.42	3.67
MWD_w(mm)	0.87	0.54	0.56
MWD_w/MWD_d	0.22	0.15	0.15
Chemical			
Total N ($g\ kg^{-1}$)	1.88^{a}	1.53^{b}	1.39^{b}
P (ppm)	4.32	3.76	2.28
K (ppm)	402^{a}	348^{ab}	281^{b}
$CaCO_3$ ($g\ kg^{-1}$)	278	287	294
pH	8.45	8.42	8.56
EC ($\mu s/cm^{-1}$)	211	230	211
Organic matter and biological			
TOC ($g\ kg^{-1}$)	17.3^{a}	14.3^{b}	10.5^{c}
C/N	9.18	9.33	7.78
POM-C ($g\ kg^{-1}$)	4.02^{a*}	3.51^{a*}	2.81^{b*}
MBC ($mg\ kg^{-1}$)	217^{a}	111^{b}	49^{c}
Earthworm($g\ m^{-2}$)	128^{b}	198^{a}	41^{c}

Values within the same row followed by a different uppercase letter are significantly different among treatments at $P<0.05$.

Table 3. Results of the analysis of variance (ANOVA) in the 5-15 cm depth for physical, chemical, organic matter and biological soil properties.

Soil properties	No-tillage	Minimum tillage	Conventional tillage
Physical			
BD ($Mg.m^{-3}$)	1.65^{a}	1.63^{ab}	1.56^{b}
PR (Mpa)	1.56^{a}	0.69^{b}	0.76^{b}
MWD_d(mm)	3.78	3.84	4.07
MWD_w(mm)	1.17	1.03	1.08
MWD_w/MWD_d	0.31	0.26	0.27
Chemical			
Total N ($g\ kg^{-1}$)	1.41^{a}	1.36^{a}	1.14^{b}
P (ppm)	2.54	2.68	2.66
K (ppm)	267	290	255
$CaCO_3$ ($g\ kg^{-1}$)	286	284	289
pH	8.66	8.51	8.59
EC ($\mu s/cm^{-1}$)	219	216	217
Organic matter and biological			
TOC ($g\ kg^{-1}$)	11.5^{a}	12.7^{a}	9.8^{b}
C/N	8.57	9.38	8.57
POM-C ($g\ kg^{-1}$)	1.89	1.97	1.22
MBC ($mg\ kg^{-1}$)	43.5	49.8	30.9

Values within the same row followed by a different uppercase letter are significantly different among treatments at $P<0.05$.

First depth (0-5 cm). The correlation matrix (determinant<0.0001) developed for the eight soil attributes selected for the 0-5 cm depth to represent soil properties showed several correlations among the variables, with significant relationships being identified for 21 of the 32 soil attribute pairs (Table 4). Several high positive correlations were found, being the highest those found among N and BD, TOC and K, TOC and MBC, and MBC and K.

Table 4. Correlation among measured soil properties considered for factor analysis in the 0-5 cm depth across all management treatments.

	BD	PR	N	K	TOC	POM-C	MBC
BD	1						
PR	0.545**	1					
N	**0.817*** **	0.567**	1				
K	0.552**	0.455*	0.514*	1			
TOC	0.467*	0.585**	0.688**	**0.786*** **	1		
POM-C	0.452*	0.204	0.242	0.601**	0.453*	1	
MBC	0.659**	0.643**	0.725**	**0.781*** **	**0.875*** **	0.591**	1
Earthworm	-0.47	-0.132	0.231	0.455*	0.594**	0.347	0.336

* Correlation is significant at P<0.10.
** Correlation is significant at P<0.05.
*** Correlation is significant at P<0.01.

A principal components analysis identified two factors (F1 and F2) with eigenvalues > 1 for the 0-5 depth. These factors explained > 76 % of variability in measured soil properties (Table 5). Considering the individual soil attributes, these two factors explained > 70% of variance for seven of the eight soil attributes. POM-C was the exception (Table 6).

Table 5. Eigenvalue proportion and cumulative variance explained by factor analysis using the correlation matrix of the standardized data of soil attributes at 0-5 cm depth.

Factors	Eigenvalue	Proportion (%)	Cumulative (%)
0-5 cm			
F1	4.69	58.7	58.7
F2	1.44	17.9	76.6

Only factors with eigenvalues >1 are shown.

Factor scores were calculated and tested for significant differences in response to tillage as in Imaz et al. 2010 (Table 6). Both F1 and F2 were sensitive to management for this soil depth. Therefore, only those soil attributes with a high proportion of variability explained by those factors were taken into account for SQI selection. The two soil attributes with highest loadings in F1 and F2 were BD and earthworm density, respectively. PR and total N had loadings for F1 within 10 % of that of BD, while TOC had the second highest loading for F1. Those attributes were selected as the most sensitive near-surface SQI for the studied soil.

Table 6. Proportion of variance explained using varimax rotation for each of the retained factors and communalities for the selected soil attributes for the 0-5 cm depth, and effect of management on factor scores for the studied management systems (P<0.05).

Soil properties	F1	F2	Communality
BD	0.869	0.148	0.777
PR	0.850	0.033	0.724
N	0.815	0.294	0.750
K	0.507	0.725	0.783
TOC	0.552	0.748	0.864
POM-C	0.277	0.639	0.485
MBC	0.726	0.607	0.895
Earthworms	-0.213	0.898	0.852
Management effect			
ANOVA *P*-value	<0.0001	<0.0001	
Mean scores			
NT	1.19[b]	0.44[b]	
MT	-0.85[a]	0.77[b]	
CT	-0.34[a]	-1.22[a]	

Values within the same column followed by a different uppercase letter are significantly different at P<0.05.

Second depth (5-15 cm). A significant correlation matrix (determinant <0.0001) was developed using four soil attributes for the 5-15 depth increment. That attributes; BD, PR, total N and TOC, were the only ones found to be sensitive to management in ANOVA analysis (Table 3). The correlation matrix identified 3 significant correlations of the 10 possible pairs at this depth, being the highest correlation found for BD and TOC (Table 7).

Table 7. Correlation among measured soil properties considered for factor analysis in the 5-15 cm depth across all management treatments.

	BD	PR	N	TOC
BD	1			
PR	0.432*	1		
N	0.271	0.351	1	
TOC	**0.592****	0.221	0.429*	1

* Correlation is significant at P<0.10.
** Correlation is significant at P<0.05.

Principal component analysis identified one factor (F3) with eigenvalues >1. This factor explained > 52 % of variability in selected

soil properties (Table 8). Factor F3 had high positive loadings (>78 %, Table 9) from BD and TOC. Scores for factor F3 were also calculated and tested for significant differences due to management. Difference for F3 were significant at P<0.05 (Table 9). BD and TOC as the two soil attributes with highest loadings for F3 can be considered sensitive SQI for this depth in the studied soil.

Table 8. Eigenvalue proportion and cumulative variance explained by factor analysis using the correlation matrix of the standardized data of soil attributes at 5-15 cm depth.

Factors	Eigenvalue	Proportion (%)	Cumulative (%)
5-15 cm			
F3	2.09	52.2	52.2

Only factors with eigenvalues >1 are shown

Table 9. Proportion of variance explained using varimax rotation for each of the retained factors and communalities for the selected soil attributes for the 5-15 cm depth, and effect of management on factor scores for the studied management systems (P<0.05).

Soil properties	F3	Communality
BD	0.805	0.648
PR	0.656	0.430
N	0.678	0.460
TOC	0.787	0.620
Management effect		
ANOVA *P*-value	<0.0001	
Mean scores		
NT	0.89[b]	
MT	0.34[b]	
CT	-1.23[a]	

Values within the same column followed by a different uppercase letter are significantly different at P<0.05.

SMAF soil quality analysis. The evaluation of soil quality was done following the Soil Management Assessment framework (SMAF) developed by Andrews et al. (2004), as an international recognized SQ assessment tool. A MDS was built from measured soil properties in this study that can be interpreted by SMAF. Several physical, chemical and biological properties that included the most sensitive

obtained indicators were selected (BD, WSA, AWHC, P, K, pH, EC, TO, and MBC). The scores obtained by the SMAF interpretation algorithms for each selected soil quality indicator are shown in Tables 9 and 10. All these scores were also integrated into an overall SQ index.

Table 10. SMAF soil quality indicator scores and the overall soil quality index (SQI) values as affected by tillage system at 0-5 cm depth.

Soil quality indicators	No-tillage	Minimum tillage	Conventional tillage
Physical			
BD	0.24^{b}	0.31^{a}	0.30^{ab}
WSA	0.55^{a}	0.40^{b}	0.38^{b}
AWHC	0.67^{a}	0.67^{a}	0.64^{a}
Chemical			
P	0.64^{a}	0.42^{ab}	0.21^{b}
K	1.00^{a}	1.00^{a}	1.00^{a}
pH	0.65^{a}	0.66^{a}	0.62^{a}
EC	1.00^{a}	1.00^{a}	1.00^{a}
Organic matter and biological			
TOC	0.96^{a}	0.89^{b}	0.62^{c}
MBC (mg kg^{-1})	0.92^{a}	0.41^{b}	0.10^{c}
SQ Index	0.76^{a}	0.69^{b}	0.62^{c}

Values within the same row followed by a different uppercase letter are significantly different at P<0.05.

In the 0-5 cm depth increment, significant differences among tillage treatments were observed in the score of five SQ indicators (Table 10). Among physical indicators, the highest differences were observed in aggregates stability; scores for WSA were higher in NT than in MT and CT. Significant differences were also observed in BD scores, being the score for NT lower than the one obtained by MT. Finally, the low BD scores obtained for all the three different tillage managements are remarkable.

P scores were significantly different among tillage treatments, obtaining NT a higher P score than CT. The scores for K, pH and EC showed no differences among treatments. Moreover, K and EC achieved scores of 1.0, indicating that these indicators were functioning at their full potential for this soil. Thus, P was within

chemical indicators the most sensitive to management. TOC and MBC scores were significantly different among treatments, being the highest scores for NT and the lowest for CT (Table 10). Finally, the values obtained for the SQ index were significantly different among the three treatments for the 0-5 cm depth (Table 10). NT obtained the highest value, followed by MT and CT.

Table 11. SMAF soil quality indicator scores and the overall soil quality index (SQI) values as affected by tillage system at 5-15 cm depth.

Soil quality indicators	No-tillage	Minimum tillage	Conventional tillage
Physical			
BD	0.24^{b}	0.25^{ab}	0.27^{a}
WSA	0.74^{a}	0.63^{a}	0.76^{a}
AWHC	0.60^{a}	0.60^{a}	0.68^{a}
Chemical			
P	0.26^{a}	0.30^{a}	0.31^{a}
K	1.00^{a}	1.00^{a}	1.00^{a}
pH	0.61^{a}	0.63^{a}	0.59^{a}
EC	1.00^{a}	1.00^{a}	1.00^{a}
Organic matter and biological			
TOC	0.71^{a}	0.79^{a}	0.55^{b}
MBC (mg kg^{-1})	0.08^{a}	0.11^{a}	0.06^{a}
SQ Index	0.64^{a}	0.65^{a}	0.64^{a}

Values within the same row followed by a different uppercase letter are significantly different at $P<0.05$.

In the 5-15 cm depth increment, only two of the SQ indicators showed significantly different scores, BD and TOC (Table 11). CT had a higher BD score than NT. As in the 0-5 cm depth increment, within physical indicators BD had low scores for the three treatments. TOC scores in this depth were lower for CT than in the other two treatments (MT and NT). Finally, NT and MT treatments achieved lower SQ index values for the 5-15 cm depth increment than those obtained for the 0-5 cm depth increment, so the differences observed in the previous depth among treatments were not observed for the 5-15 depth increment.

DISCUSSION

Soil quality indicators selection, dryland soil vs irrigated soil. The analysis of soil parameters in the third season after conversion to irrigation allowed to evaluate the effect of such a land use change over soil parameters, taking as a reference the SQ indicators studied previously in dryland conditions in this site (Bescansa et al., 2006; Virto et al., 2007; Imaz et al., 2010).

Several of the soil parameters sensitive to management in dryland conditions remained so three years after the adoption of irrigation. In particular, the two physical indicators BD and PR remained sensitive in both depth increments. Total N, TOC and earthworm density remained sensitive to management in the 0-5 cm depth increment. However, some remarkable differences in comparison with dryland conditions were found. Differences in POM among managements, which were significant and determinant to denote POM as the indicator most sensitive to management in dryland conditions (Imaz et al., 2010), had disappeared with the adoption of irrigation. Under irrigation, differences were only found in the 0-5 cm depth at $P<0.1$ (Table 2). Aggregates stability was also a sensitive parameter in dryland conditions, important for explaining differences among managements. Without irrigation, NT showed higher aggregates stability than MT and CT. However, aggregates stability expressed as MWD was reduced in NT management after irrigation adoption and differences among treatments were not found in the new conditions. However, when expressed as WSA, significant differences among NT (16±0 %) and both MT (12±2 %) and CT (12±2 %), were found. WSA values were therefore included in the SMAF SQ analysis. Although it was not included in the PCA, as it was not included in the previous study from Imaz et al. (2010) in dryland conditions. Finally, within chemical parameters, K (0-5 cm depth) and N (5-15 cm depth) also disappeared as sensitive parameters in irrigation conditions.

Consistent with the changes observed in soil parameters sensitivity to management, the results of the selection of SQ indicators varied from those obtained in dryland conditions. As described above, POM disappeared as an appropriate indicator in the

new water regime conditions, while several soil indicators remained as adequate indicators for the 0-5 cm depth increment (BD, PR, TOC and earthworm density). Moreover, in the 5-15 cm depth increment, POM and aggregates stability (selected as the most sensitive SQ indicators for this depth in dryland conditions), were replaced by BD and TOC, maintaining however a physical and an organic matter parameter as the most sensitive indicators. These changes highlight the necessity of reevaluating SQ indicators when the conditions of the agroecosystems are changed. This confirms the observation of Andrews et al. (2001) that MDS indicators may need to change with differing management climate, soil type, or even time. This is in fact in accordance with the current SQ paradigm, which is based in the evaluation of the capacity of a soil to function within the limits of a determined ecosystem (Doran and Parkin, 1994). In addition, the short time period in which changes have occurred ratify the precocity of some of the previously selected indicators (e.g. POM). Particulate organic matter has been highlighted in bibliography as a rapid and selective soil quality indicator for land use and soil management changes (Cambardella and Elliot, 1993; Jastrow et al., 1996; Marquez et al., 1999).

SMAF soil quality index and evaluation of managements. The observed differences among treatments within physical indicators, showed an increase in soil compaction along the profile in NT management, expressed by lower BD scores. This effect of NT has been previously observed in several studies (e.g. Mahboubi et al., 1993; Lampurlanés and Cantero-Martínez, 2003). Moreover, the low BD scores obtained by the three different managements indicate that the studied soil was not functioning at its full potential due to a possible compaction problem according to SMAF interpretation. In contrast, the higher aggregates stability expressed as WSA in NT for the 0-5 cm depth increment revealed a better structure of the soil for that management. Previous studies that used SMAF as a SQ index have also reported the sensitivity of WSA (Karlen et al., 2006; Jokela et al., 2009) and BD (Jokela et al., 2009; Karlen et al., 2011) as SQ indicators, and observed score differences among soil managements for those parameters.

Increased WSA did not have an evident effect on the soil water retention characteristics (no differences were found in AWHC scores), but the overall scores for physical indicators in SMAF were higher for NT than for MT and CT (Table 10). Improved aggregation has been previously observed in NT systems in the Mediterranean region (Marbet et al., 2001; Alvaro-Fuentes et al., 2007; Álvaro-Fuentes et al., 2008), and has been generally associated with higher levels of TOC and organic matter stability (Álvaro-Fuentes et al., 2009).

The scores obtained for chemical indicators showed differences among treatments only in one indicator (P) for the 0-5 cm depth increment. P was not included in the MDS obtained after PCA because differences among managements were not significant. However, when interpreted by SMAF algorithms, the scores obtained for P values were significantly different, because the observed values of P were low, and located in the exponential increase region of the interpretation curve. The low P score obtained by CT indicated that it may be limiting for soil functioning, while the score was notably higher for NT management indicating an improved chemical soil functioning under that management. In calcareous soils as the one studied here, a low availability of P is common due to its retention by carbonates (Brady and Weil, 2002). Small increments in this parameter can therefore be very significant in terms of crop productivity and soil biological activity. In our study, the higher score for P observed in NT for the 0-5 cm depth increment could for instance partially explain the observed higher microbial biomass. An observation supporting this hypothesis is that in the second depth, no differences were observed in the scores for both P and microbial activity among treatments. Form the point of view of chemical fertility, the observed score of 1 for K (a nutrient not added in fertilizer applied to this field) in the three management systems (Tables 10 and 11) means that the natural abundance in this soil can be considered high enough for functioning at its full potential. Therefore, the differences observed among managements in available K contents (Table 2) disappeared when these values were interpreted by SMAF, as all values performed over the maximum established by SMAF.

For both organic and biological indicators (TOC and MBC), the obtained scores also suggested that they were functioning near the full potential provided by SMAF for this soil under NT in the 0-5 cm depth increment. In contrast, the scores achieved in CT were low (near 0 in the case of MBC) indicating that the soil under this management was depleted in organic matter and its microbial community was very likely reduced and unable to accomplish at least some of their functions. Intermediate scores were achieved under MT. In the 5-15 cm depth increment, TOC scores were smaller than in the 0-5 cm depth for NT and MT managements, although the scores of both managements were higher than for CT. These results evidenced that organic matter mineralization promoted by moldboard plowing may induce a reduction of soil organic carbon that can affect soil functioning also at this depth. It is also remarkable how MBC scores dramatically decreased in all managements in comparison with the 0-5 cm depth increment. Scores for the three studied managements were near zero, revealing the reduced presence of microorganism below the first 5 cm in the studied soil. The importance of TOC as a SQ indicator is in accordance with previous studies using SMAF, which showed high TOC score differences among managements (Jokela et al., 2009; Karlen et al., 2011). TOC also achieved low scores in several of the soils evaluated through SMAF (Karlen et al., 2006; Jokela et al., 2009; Stott et al., 2011) indicating that agricultural soils are frequently depleted in organic matter. Therefore TOC is an interesting indicator to detect agricultural soil-improving managements.

The general SQ index calculated by SMAF was significantly different among managements at 0-5 cm, but not at the 5-15 cm. In the upper soil layer, NT had overall greater SQ scores than CT, and MT had an intermediate result. Data shown in Table 10 indicate that the gain in this index under NT was mostly due to better scores in aggregates stability, P and TOC contents, and a higher MBC. Comparing these results with other studies is complicated, as SQ evaluation indexes need to be compared on the basis of similar calculation methods. A recent study by Karlen et al. (2013) also evaluated different long-term tillage managements using SMAF in

dryland conditions in Iowa. Despite the differences in agronomic and climate conditions, they observed, in accordance with our results the lowest scores in soils under moldboard tillage, which had the most negative impact on several SQ indicators. Also in agreement with our observations WSA, TOC and MBC were the indicators with the highest differences among managements. In the second soil depth (5-15 cm), both P content and MBC decreased dramatically under NT in the current study. Consequently, differences among treatments in the SQ index provided by SMAF were not found in that depth increment.

Adequacy of SMAF. The overall performance achieved in this soil evaluated by SMAF indicated that it was functioning between 62 and 76 % of its potential in the 0-5 depth and between 64 and 65% in the 5-15 depth. Such results are low in comparison with the results found in the bibliography from previous soil quality studies using SMAF analysis. For example, Karlen et al. (2006) evaluated different crop rotations in a study carried out in three different sites located in Iowa and Wisconsin. They found scores higher than 0.90, 0.80 and 0.75 in each site for the 0-15 cm soil depth. Stott et al. (2011) studied different canopy development areas in several soils along the Iowa River's South Fork Watershed. They found average SMAF scores of 0.86 and 0.82 for well-developed and poorly-developed canopy areas, respectively, in the 0-10 cm soil depth. In studies where soil sampling were divided in different soil depth increments, average SMAF scores obtained for the 0-5 cm soil depth were always over 0.80 (Jokela et al., 2009; Karlen et al., 2011; Karlen et al., 2013). In our results, the scores obtained in the 5-15 cm depth were lower than in 0-5 in all cases. This was also observed in previous studies, although our values remained in general smaller than those reported elsewhere (Jokela et al., 2009; Karlen et al., 2013). The scores for the 5-15 cm depth obtained in Karlen et al. 2011 (0.53-0.69), were the only ones within the same range of those obtained in the current study. All the studies mentioned were performed in soils located in the U.S. Midwest, and comparisons with results in our conditions must be carefully considered. However, the systematically low values obtained in our study, in a soil that is irrigated and therefore achieves high yield standards (see below) suggest that, despite the flexibility of SMAF to

adapt its algorithms to different soils and ecosystem conditions, a calibration of the tool for different regional situation is probably needed. This would help to validate the scores given by SMAF, and whether they are actually answering to the real functioning capacity of the different agro-ecosystems. Nevertheless, it should be highlighted that, despite these limitations, SMAF has demonstrated to be effective for comparing soil managements in semi-arid irrigated Mediterranean soils, as the scores generally showed significant differences in many SMAF indicators, and which were sensitive enough to differentiate SQ across managements. Finally, crop yields were also recorded as an indicator of soil functioning taking into account that sustaining plant productivity is considered the major soil function for irrigated agricultural soils in the study area. Table 1 shows there were no differences among managements in average yields for the studied period. Therefore, the observed differences in SQ indicators and SQ indexes among managements did not have a detectable effect on the soil productivity function. This can probably be explained by the fact that external inputs (fertilizers and irrigation water) could have minimized soil effects limiting crop productivity. This is in contrast with yield differences found in dryland conditions in this site (Bescansa et al., 2006). Since differences in AWHC were considered the main advantage of NT in dryland conditions for crop productivity without irrigation (Bescansa et al., 2006), the implementation of irrigation, which greatly reduces the importance of this advantage and seems to have resulted in no differences in AWHC among soil management systems after three years of irrigation, can be very likely related to this absence of yield differences.

CONCLUSIONS

Factor analysis proved to be useful for selection of appropriate SQI for Mediterranean semi-arid irrigated cropland, and confirmed the necessity of reevaluating SQ indicators when the agroecosystem conditions are modified. The adoption of irrigation in a soil previously cultivated under dryland conditions resulted in a change of the main SQ indicators after just three years. Such a fast change ratifies the precocity of some of the previously selected indicators (e.g. POM).

The results obtained by the use of SMAF revealed that this framework resulted sensitive to the observed differences in SQ among soil tillage managements, and were therefore adequate to evaluate SQ differences among them. Even though, a detailed analysis of some of the interpretation algorithms seems necessary to adapt the SMAF to the local agroclimatic conditions. This seems especially important in those parameters for which systematic low values where obtained (e.g. BD).

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Chapter VI

Discusión general

Conclusiones

CHAPTER VI

Discusión general y conclusiones

Contenidos

DISCUSIÓN GENERAL

Factores reguladores de la dinámica de la materia orgánica y su estabilización en suelos cultivados, calcáreos, mediterráneos en el contexto de conversión a regadío en el valle del Ebro.

Los principales mecanismos responsables de la estabilización de la materia orgánica en el suelo han sido ampliamente descritos en la bibliografía: protección física frente a los organismos descomponedores, estabilización química mediante interacciones con la matriz mineral del suelo, y en algunos casos, recalcitrancia bioquímica de las moléculas orgánicas (Six et al., 2002; Lützow et al., 2006; Jastrow et al., 2007; Kögel-Knabner et al., 2008). A pesar de este consenso general en cuanto a los principales mecanismos estabilizadores, existen un gran número de factores y procesos involucrados y actuando al mismo tiempo que los condicionan, lo cual dificulta desentrañar la relevancia individual de cada mecanismo (Lützow et al., 2006). Además, las diferencias entre tipos de suelos y zonas agroclimáticas pueden ser importantes. En el trabajo realizado durante la presente tesis se han estudiado diversos factores tanto intrínsecos (debidos a características del propio suelo), como extrínsecos (debidos al uso o manejo de ese suelo). Además, esto se ha realizado en el contexto de transformación a regadío de una extensa superficie agrícola a nivel regional: por un lado el monitoreo de parcelas en distintas zonas agroclimáticas de la región permitió evaluar de manera general los factores de estabilización a escala regional y el trabajo a nivel de parcela experimental permitió profundizar en el efecto de factores específicos, contenido de carbonatos y laboreo, así como estudiar el efecto de la introducción del regadío *per se*.

El efecto de la transformación al regadío de tierras de secano sobre la calidad del suelo, sobre su funcionamiento y sobre la dinámica de la materia orgánica, ha sido el objeto de muy pocos estudios científicos. Sólo en esta última década algunos equipos de investigación han comenzado a prestar atención a este tema (Amos et al. 2005; Follett et al., 2005; Verma et al., 2005; Adviento-Borbe et al., 2007; Gilabell et al., 2007; Nunes et al., 2007; Denef et al., 2008). Todos los autores mencionados coinciden en que el regadío puede

ser una práctica adecuada para la mejora de la calidad del suelo y el incremento del contenido de carbono orgánico, o no, según diversos factores edáficos, climáticos y del agrosistema. Esto es función por lo tanto de parámetros como el tipo de fertilización, el tipo de rotación, y el tipo de suelo y su manejo, y por ello es necesario realizar estudios locales y a nivel de campo para evaluar con la precisión necesaria su efecto en las diferentes regiones del planeta y su impacto a nivel global. Los resultados obtenidos en el presente trabajo mediante el monitoreo realizado en distintas zonas de Navarra 5, 7 y 9 años después de dicha transformación (Capítulo I, estudio de caso), indican que el regadío ha propiciado un aumento del contenido de carbono orgánico de estos suelos respecto a su contenido en secano. Por un lado, la medición del carbono orgánico en una de las tres zonas (Funes), en una parcela adyacente que permanece en secano, resultó en un contenido de carbono orgánico de 24,9 Mg C ha^{-1} respecto a los 44,3 Mg C ha^{-1} obtenidos en regadío para esa misma zona en el horizonte labrado. En siete años, este valor correspondería a una tasa anual de 2,77 Mg C ha^{-1} en este suelo. Además, los resultados obtenidos para las tres zonas de regadío se situaron entre 30 y 60 Mg C ha^{-1}, por encima en todos los casos de los contenidos en otras regiones semiáridas en suelos no regados (West y Post, 2002). En comparación con estudios realizados en la región sobre el efecto de otras prácticas de manejo en el aumento del contenido de C orgánico en el suelo (Bescansa et al., 2006; Virto et al., 2007; Fernández-Ugalde et al., 2009), la aplicación del no laboreo en secano arrojó ganancias de entre 3,5 y 8,5 Mg ha^{-1} en el horizonte labrado en ensayos de entre 5 y 10 años de duración, respectivamente. A nivel de la Península Ibérica, González-Sánchez et al. (2012), muestran resultados muy variables de esta estrategia de manejo (entre 0 y 2,1 Mg ha^{-1} $año^{-1}$ en esta misma profundidad, para ensayos de más de 5 años de duración). La introducción del regadío, en este caso, aparece como responsable de ganancias equivalentes o mayores a las observadas con estas técnicas de manejo. Un estudio realizado a nivel nacional en Francia (ADEME, 2014), otorga a estrategias de cambio de manejo en cultivos y praderas (reducción del laboreo, gestión de los pastos, aportes de enmiendas,

implantación de cubiertas permanentes) tasas anuales de entre 0,10 y 0,50 Mg ha^{-1}.

Sin embargo, el estudio realizado a nivel de parcela experimental (Capítulo II), en el que se realizó un monitoreo en detalle de la evolución de la dinámica de la materia orgánica desde el momento de su puesta en regadío, no aportó evidencias acerca de que dicha transformación supusiera una acumulación de carbono orgánico en los dos primeros años tras la transformación. A pesar de ello, se espera que en un plazo de tiempo más largo se constaten cambios en este sentido, ya que según comprobó Denef et al. (2008) en agrosistemas similares al nuestro, tras décadas de cultivo en regadío, el cambio en el contenido de C orgánico del suelo había sido pequeño en relación al gran aumento de las entradas de C orgánico. Nuestros resultados indican que podría ser difícil observar en el corto plazo cambios significativos en el contenido total de C orgánico del suelo. Este menor incremento del contenido de C orgánico respecto al incremento en la incorporación de residuos vegetales, puede explicarse por el aumento de la actividad microbiana, asociado al cambio en las condiciones ambientales del suelo en regadío, que aumentaría la tasa de mineralización de la materia orgánica (Jackson et al., 2003; Martens et al., 2005). Además, Denef et al. (2008) también observaron un menor cambio del contenido de C orgánico en suelos manejados con laboreos intensivos como los llevados a cabo en la parcela experimental, lo que podría causar que la obtención de cambios medibles en el contenido de C orgánico se retrase en ese caso.

Por otro lado, el estudio de la dinámica de incorporación de la materia orgánica a través de herramientas de análisis isotópico, si evidenció en el corto plazo una aceleración del ciclo de incorporación de materia orgánica en el suelo. El análisis isotópico permitió contabilizar el carbono orgánico proveniente de restos de maíz cultivado por primera vez en la parcela durante los dos años de estudio. En regadío el carbono orgánico de origen maíz fue el 13,4 % del carbono orgánico total mientras que en secano supuso el 4,9 %. La capacidad de detección de cambios en el corto plazo de las

herramientas de análisis isotópico ya había sido observada con anterioridad (Urbanek et al., 2011).

La interacción entre la materia orgánica incorporada y la estructura física del suelo también fue estudiada en esta misma parcela experimental (Capítulo II), con el objetivo de entender mejor la dinámica de estabilización de la materia orgánica. Su incorporación a la estructura del suelo siguió un orden jerárquico (Six et al., 1999), incorporándose primeramente en las fracciones de mayor tamaño (macroagregados > 250 µm). Además, la mayor concentración de C orgánico total presente en los macroagregados respecto al resto de fracciones del suelo (microagregados y fracción mineral), sugiere que la materia orgánica actúa como agente de unión en los macroagregados estables en agua en este suelo, de acuerdo al modelo jerárquico de agregación (Puget et al., 2000). Sin embargo, no se observaron efectos en la agregación que indicaran que el cambio en el ciclo de la materia orgánica hubiera propiciado un aumento en los niveles de macroagregación ni microagregación, tanto intra-macroagregados como microagregados libres. Estos resultados contrastan con los obtenidos en estudios llevados a cabo acerca de la influencia del regadío en la dinámica de la agregación y la materia orgánica en el suelo en otras regiones, como el de Gillabel et al. (2007) tras más de 30 años de cultivo en regadío en una zona semiárida de Nebraska. Este estudio evidenció que el modelo jerárquico no se cumplía en esas condiciones, y fue necesario el planteamiento de un modelo distinto que explicara la dinámica de agregación y de la materia orgánica seguidas en esos suelos. El modelo propuesto se basó en que el aumento de entradas de materia orgánica junto con el aumento de la actividad microbiana, provocan una aceleración en la formación de microagregados libres (no formados en el seno de macroagregados), en los que la materia orgánica fresca quedaría retenida directamente, sin la necesidad de la formación previa de macroagregados estables, que sirvieran como espacio para la formación de nuevos microagregados. En nuestro trabajo, a pesar de la similitud de las condiciones, no hubo resultados que indicaran una dinámica de este tipo, ya que no se produjo un aumento de la microagregación libre ni de la concentración de C

orgánico en los microagregados. Además, a partir del seguimiento isotópico del carbono orgánico se observó que la incorporación de la materia orgánica nueva, de origen maíz, tuvo lugar preferentemente en los macroagregados. Estos resultados permiten pensar que en un futuro la dinámica de la materia orgánica y la de agregación se comporten según el modelo jerárquico (Six et al., 1999), aunque estudios realizados en suelos similares (Fernández-Ugalde et al., 2011; Fernández-Ugalde et al., 2014) también sugieren que la relación entre la dinámica de incorporación de la materia orgánica y la agregación no tiene una relación tan directa en suelos con altas concentraciones de carbonatos, como es el caso. Esta interacción se explica en detalle más adelante, al haber sido objeto específico de estudio en esta tesis.

En cuanto a la interacción que diversos factores pueden tener sobre la estabilización de la materia orgánica tras la puesta en regadío, el monitoreo de parcelas a nivel regional mostró resultados interesantes de su efecto a esa escala (Capítulo I, estudio de caso). No se observaron diferencias en contenido de carbono orgánico entre las tres zonas estudiadas debido al gradiente de aridez que existe entre ellas, resultando por lo tanto, en este caso, nulo el efecto del clima. Por otro lado, el contenido de carbono evaluado en relación a la rotación de cultivos tampoco mostró diferencias significativas. Resultado que coincide con la comparación realizada entre trigo y maíz a nivel de parcela experimental (Capítulo II), donde a pesar de las notables diferencias en cuanto a restos de cosecha incorporados en el suelo entre estos cultivos, no se obtuvieron diferencias en contenido de carbono orgánico en el suelo en los dos primeros años. Finalmente, el único factor que mostró una correlación positiva con el contenido de carbono orgánico a lo largo de las tres zonas de estudio fue el contenido de arcilla del suelo. Esta relación entre el contenido en arcilla y la estabilización de la materia orgánica en el suelo está bien establecida en la bibliografía. El contenido de arcilla tiene una relación directa con la estabilización química de la materia orgánica, debido a la formación de complejos organominerales (Hassink, 1997, Six et al., 2002). Además el contenido de arcilla también ha sido reconocido como un factor importante en la formación y estabilización

de los agregados del suelo (Six et al., 2004; Denef y Six, 2005; Denef y Six, 2006). De esta manera también favorece indirectamente la estabilización física de la materia orgánica que queda protegida en el interior de los agregados (Denef et al., 2004). En conclusión, a nivel regional la interacción de los otros dos factores considerados (clima y rotación de cultivo) con el cambio a regadío, no fue determinante en la estabilización de la materia orgánica. Siendo, en cambio, las características intrínsecas del propio suelo (contenido de arcilla) aquellas que influyeron en mayor medida en la cantidad de materia orgánica estabilizada tras la conversión a regadío.

Por otro lado, el laboreo es considerado un factor extrínseco muy relevante en los procesos de estabilización de la materia orgánica en el suelo (West and Post, 2002; Franzluebbers, 2005; González-Sánchez et al., 2012), ya que es determinante en el proceso de incorporación y mineralización de la materia orgánica en suelos agrícolas (Singer and Munns, 2006; Singh et al., 2007). Por ello en el presente trabajo ha sido objeto de un estudio detallado a nivel de parcela experimental. En concreto, se estudió su interacción con la puesta en marcha del sistema de regadío, en un ensayo a largo plazo de agricultura de conservación (Capítulo I, estudio de caso). Los resultados obtenidos corroboraron que tras la implantación del regadío en nuestras condiciones edafoclimáticas, los sistemas de no laboreo fueron más efectivos en relación a la estabilización de la materia orgánica en el suelo. Recientemente en un estudio llevado a cabo en Estados Unidos en parcelas de maíz cultivado en regadío, Follet et al. (2013) observaron también una mayor retención de materia orgánica recientemente incorporada y una menor mineralización de materia orgánica heredada en el suelo en sistema de no laboreo respecto a laboreo convencional. Estos resultados muestran que a pesar de que el no laboreo en regadío no representa una ventaja frente al laboreo en cuanto a producción de biomasa vegetal, como se había observado previamente en condiciones de secano semiárido (Bescansa et al., 2006; Imaz et al., 2010), puede ser efectivo en la estabilización de la materia orgánica. Resultados obtenidos en nuestro estudio en cuanto a la evolución de la agregación en ambos sistemas, indican que la potenciación de los

mecanismos de protección física de la materia orgánica en el suelo, pueden ser la causa principal de su estabilización bajo sistemas de no laboreo.

Por último, el contenido de carbonatos ha sido también estudiado en el presente trabajo (Capítulo IV) de manera específica, como factor de estabilización de la materia orgánica, al considerarse que puede tener gran importancia en el funcionamiento del suelo en nuestras condiciones edafoclimáticas. En diversas zonas de las regiones semiáridas del planeta, debido a las condiciones climáticas y al material parental presente, es común encontrar importantes contenidos de carbonatos geogénicos en los suelos. Concretamente, en España, los Calcisoles son uno de los grupos de suelos más frecuentes (European Comission, 2005). El contenido de carbonatos alcalinotérreos confiere a estos suelos características específicas que pueden tener una importante influencia en su calidad física y en la dinámica de la materia orgánica. Los primeros estudios sobre el efecto del carbonato cálcico sobre la estabilización de la materia orgánica ligaron este proceso a interacciones químicas con los cationes Ca^{2+}. Sin embargo, ya en el año 2000, Baldock y Skjemstad relacionaron la estabilización de la materia orgánica en estos suelos a sus propiedades físicas diferenciadas: disponibilidad de agua y oxígeno, protección física frente a organismos descomponedores y dinámica de la agregación. Estos mecanismos propuestos por Baldock y Skjemstad concuerdan con la teoría de la "regulatory gate", planteada por Kemmit et al. (2008). Según la citada teoría, son estos procesos abióticos los que actúan como limitantes de la mineralización de la materia orgánica en el suelo, siendo los procesos microbianos y las características de la materia orgánica, factores reguladores de la mineralización menos relevantes.

En nuestras condiciones agroclimáticas, en Rodezno (La Rioja) se ha llevado a cabo en los últimos años el estudio de dos suelos (*Calcixerept Típico* y *Haploxerept Cálcico*; Soil Survey Staff, 2006) pertenecientes a una misma parcela agrícola, y por lo tanto bajo el mismo manejo, diferenciados en su fracción mineral, principalmente en su contenido en carbonatos alcalinotérreos. Los primeros resultados confirmaron diferencias en la dinámica de la agregación

entre suelos carbonatados y no carbonatados (Fernández-Ugalde et al., 2011). Se observó como en el suelo carbonatado la dinámica de la agregación era menos dependiente de la materia orgánica debido a la intervención de los carbonatos, lo cual podría propiciar una mayor estabilidad de los agregados. Sin embargo, el estudio de la mineralogía de los agregados no confirmó estas diferencias (Fernández-Ugalde et al., 2014). Por otro lado, mediante el análisis de láminas delgadas se confirmó la distinta estructura física de los agregados, que en el suelo carbonatado se mostró más masiva y con gran presencia de cristales de calcio (Virto et al, 2013).

El estudio llevado a cabo en esta tesis, sobre muestras de este mismo par de suelos en Rodezno, bajo la hipótesis de que las diferencias en las características físicas del suelo carbonatado son responsables de limitaciones en la mineralización de la materia orgánica y por lo tanto favorecen su estabilización, confirmó la existencia de diferencias físicas entre ambos suelos y una menor tasa de mineralización de la materia orgánica en el suelo carbonatado, así como un mayor contenido de carbono orgánico total (Capítulo III). Sin embargo, el estudio no mostró evidencias respecto al papel de las características físicas del suelo carbonatado como limitantes de la mineralización. En primer lugar, las diferencias en cuanto a las características físicas se concretaron únicamente en una distinta distribución de tamaños de los microporos, pero no hubo diferencias ni en la porosidad total ni en la capacidad de retención de agua disponible entre ambos suelos. Además, esta distinta distribución de la porosidad resultó en un ambiente más favorable para la mineralización en el suelo carbonatado, debido a la mayor presencia de microporos de volúmenes altos entre los rangos estudiados. Por último, los resultados de las incubaciones de suelo realizadas confirmaron una mayor protección física en la estructura de los macroagregados en el suelo no carbonatado. Este aspecto quedó confirmado al observarse un mayor incremento de la respiración en el suelo no carbonatado tras destruir la estructura del mismo. Por lo tanto, otros mecanismos de estabilización de la materia orgánica en el suelo deben ser responsables de la mayor protección observada en el suelo carbonatado. Concretamente, diferencias en las

características bioquímicas de la materia orgánica estabilizada pueden ser el origen de esta estabilización y deben ser analizadas en futuros estudios. Baldock y Skjemstad (2000) ya resaltaron la presencia de cationes de Ca en las moléculas orgánicas de los suelos calcáreos, que pueden resultar en uniones entre los polímeros orgánicos (Ojeda et al., 2003). Además, las características de la materia orgánica heredada pueden determinar la cantidad y calidad de la materia orgánica presente en el suelo después de largos periodos de tiempo tras ser cultivada (Plante et al., 2010).

La evaluación de la calidad del suelo como herramienta para el estudio de suelos de clima mediterráneo semiárido en condiciones de regadío

La evaluación de la calidad de suelos es fundamental para el manejo sostenible de los mismos. Para ello es necesario contar con una serie de indicadores de calidad de suelo adecuados a las condiciones del ecosistema en que se sitúan (Doran y Parkin, 1994; Andrews et al., 2001). Además, para que estos indicadores resulten eficaces en su estudio a nivel local es interesante contar con índices de calidad de suelos adaptados y correctamente calibrados para su uso en las condiciones edáficas, climáticas y de manejo de cada contexto. Los resultados obtenidos en el capítulo V de la presente tesis han permitido avanzar en el conocimiento sobre estas cuestiones en nuestra región.

En primer lugar, el análisis de un conjunto de indicadores preseleccionados de suelo en un estudio sobre técnicas de manejo en un ensayo de larga duración en secano (Bescansa et al., 2006; Virto et al., 2007; Imaz et al., 2010), tres campañas después de su transformación a regadío, permitió evaluar la sensibilidad de dichos indicadores al efecto de este cambio, y su adecuación como indicadores de calidad en las nuevas condiciones de regadío. Varios de los parámetros de suelo sensibles al manejo en condiciones de secano, mantuvieron dicha sensibilidad tras la implantación del regadío. Concretamente, dos parámetros físicos, densidad aparente (Da) y resistencia a la penetración (RP) continuaron siendo sensibles

al manejo en las dos profundidades estudiadas. El nitrógeno total (NT), el carbono orgánico total (COT) y la densidad de lombrices permanecieron siendo sensibles al manejo, pero únicamente en superficie (0-5 cm). Por otro lado, se observaron algunas diferencias destacables respecto a las condiciones de secano. Las diferencias entre tratamientos encontradas en secano en el contenido de materia orgánica particulada (MOP) fueron determinantes y dieron como resultado que este parámetro fuera seleccionado como el indicador más sensible (Imaz et al., 2010). En cambio, tras tres años en regadío, estas diferencias habían desaparecido, y por lo tanto, la idoneidad de este parámetro como indicador de la calidad del suelo, también. También se observaron algunos cambios menos relevantes en los indicadores de estabilidad de la estructura. Finalmente, entre los parámetros químicos, el potasio (K) en la profundidad 0-5 cm y el nitrógeno (N) en 5-15 cm también pasaron de ser sensibles al manejo en condiciones de secano a no serlo en regadío.

En consonancia con los cambios anteriores, los resultados obtenidos en la selección de indicadores de calidad de suelo fueron diferentes a aquellos que se obtuvieron anteriormente en condiciones de secano. Como ya se ha comentado, la MOP no resultó un indicador de calidad de suelo apropiado en condiciones de regadío. En cambio varios de los indicadores seleccionados en secano sí se mantuvieron también en condiciones de regadío para la profundidad 0-5 cm (Da, RP, COT y densidad de lombrices). Además, en la profundidad 5-15 cm COT y Da fueron seleccionados como los indicadores más sensibles a los distintos manejos, a diferencia que en las condiciones de secano, en las que se seleccionó a la MOP y la estabilidad de los agregados para dicha profundidad. Estos cambios subrayan la necesidad de una reevaluación de los indicadores de calidad de suelo cuando se modifican las condiciones en el ecosistema agrícola. Y confirman la observación realizada por Andrews et al. (2001), según la cual los indicadores de calidad de suelo más adecuados podrían variar según el uso o manejo del suelo, el clima, el tipo de suelo e incluso el tiempo. De hecho, esto resulta coherente con el actual concepto de calidad de suelo, el cual se basa en la capacidad de un suelo para funcionar dentro de los límites de

un ecosistema (Doran y Parkin, 1994). Por otro lado, el breve periodo en que estos cambios han ocurrido confirma la precocidad con que responden a los cambios algunos de los parámetros previamente seleccionados como indicadores (MOP). La materia orgánica particulada ha sido destacada en la bibliografía como un indicador de calidad de suelo rápido y sensible a los cambios de uso y manejo del suelo (Cambardella y Elliot, 1993; Jastrow et al., 1996; Marquez et al., 1999). Su significación parece ser menor en el caso de suelos regados en la región, o al menos en relación a las diferencias inducidas por el laboreo en este tipo de suelos.

En segundo lugar, la evaluación de los distintos manejos existentes en el ensayo (no laboreo (NL), laboreo convencional (LC) y laboreo mínimo (LM)), realizada a través de un marco conceptual que permite deducir un índice cuantitativo de calidad de suelo (Soil Management Assessment Framework, SMAF) también tuvo resultados que permitieron al mismo tiempo evaluar la calidad del suelo en el ensayo anterior, y la adecuación de esta metodología, tal y como está descrita originalmente, a su uso en las condiciones agroclimáticas de nuestra región. Las diferencias observadas entre tratamientos en cuanto a la valoración obtenida en SMAF de los indicadores físicos, mostraron un aumento de la compactación a lo largo del perfil del suelo en el NL, expresado mediante la obtención de valores menores en SMAF para el parámetro Da. Este efecto del NL ya había sido observado previamente en diversos estudios (Mahboubi et al., 1993; Lampurlanés y Cantero-Martínez, 2003). Además, las bajas puntuaciones en Da obtenidos en los tres manejos indican que el suelo estudiado no está funcionando a su máximo potencial debido a una excesiva compactación según la valoración realizada por SMAF. En cambio, el suelo bajo NL obtuvo valoraciones más altas para la estabilidad de los agregados (cantidad de agregados estables al agua) en la profundidad de 0-5 cm, indicando una mejor estructura del suelo en este manejo. Estudios previos que han utilizado SMAF como índice de calidad del suelo, también han obtenido una alta sensibilidad de los parámetros estabilidad de los agregados (Karlen et al., 2006; Jokela et al., 2009) y Da (Jokela et al., 2009; Karlen et al., 2011) como indicadores de calidad del suelo, y

han observado diferencias significativas entre los valores obtenidos por estos parámetros para distintos manejos. En el estudio presentado en el capítulo V, a pesar de que la mayor cantidad de agregados estables al agua encontrados en el no laboreo no tuvo un efecto evidente en la capacidad de retención de agua del suelo, la puntuación general para los indicadores físicos otorgada por SMAF fue mayor para el manejo NL que para los manejos con laboreo, ML y CL. Un incremento en la agregación como resultado del uso del no laboreo había sido previamente observada en estudios realizados en la región mediterránea (Marbet et al., 2001; Álvaro-Fuentes et al., 2007; Álvaro-Fuentes et al., 2008), y ha sido relacionada con mayores niveles de COT y mayor estabilidad de la materia orgánica (Álvaro-Fuentes et al., 2009).

Las puntuaciones obtenidas para los indicadores químicos en los primeros 5 cm de suelo sólo mostraron diferencias significativas entre manejos en el caso del fósforo (P). Este parámetro no había sido incluido en la lista de indicadores tras el análisis de componentes principales (ACP), ya que no mostró diferencias significativas entre tratamientos. Sin embargo, las puntuaciones obtenidas a través de los algoritmos de interpretación de SMAF fueron significativamente diferentes. Esto es así debido a que los valores de P para este suelo son bajos y pequeños incrementos en su contenido resultan en aumentos significativos de su puntuación en SMAF. La baja puntuación de P obtenida en el tratamiento LC indica que podría estar limitando el funcionamiento del suelo en este manejo, mientras que la puntuación en NL fue mayor indicando una mejora en el funcionamiento químico del suelo bajo este manejo.

Desde el punto de vista de la fertilidad química, la máxima puntuación obtenida para K en los tres manejos (nutriente que no se aporta en el abonado) significa que la abundancia natural de este elemento en el suelo estudiado es suficiente para alcanzar su máximo potencial de funcionamiento. Por lo tanto, aunque existían diferencias significativas en el contenido de K entre los tres manejos, estas diferencias desaparecieron según la evaluación y puntuación de este parámetro realizada según los algoritmos de SMAF.

Los dos indicadores relacionados directamente con la fracción orgánica evaluados con SMAF, COT y CBM, obtuvieron puntuaciones altas en SMAF para el tratamiento NL, indicando que están cerca del potencial máximo de funcionamiento para este manejo en la profundidad 0-5 cm. Contrariamente, las puntuaciones obtenidas para el tratamiento LC fueron muy bajas (cerca de 0 para CBM). Por lo tanto, podemos decir que bajo este manejo el suelo bajo manejo convencional se mostró lejos de su potencial máximo en relación a estos parámetros. Resultados intermedios para ambos indicadores se obtuvieron en el tratamiento ML. En la profundidad de 5-15 cm, las puntuaciones de COT fueron en todos los casos menores a aquellas obtenidas en la profundidad superficial, y fue el manejo LC el que obtuvo la menor puntuación. Estos resultados evidenciaron que la mineralización de la materia orgánica favorecida por el laboreo con vertedera, puede provocar una reducción del carbono orgánico en el suelo, que puede afectar su funcionamiento también para esta profundidad. La importancia de COT como indicador de la calidad de suelo también ha sido observada en estudios previos que han utilizado SMAF (Jokela et al., 2009; Karlen et al., 2011), observándose diferencias altas entre las puntuaciones obtenidas por los distintos tratamientos. Además, en varios de los suelos evaluados mediante SMAF, las puntuaciones obtenidas por COT han sido bajas (Karlen et al., 2006; Jokela et al., 2009; Stott et al., 2010) indicando que es habitual que los suelos agrícolas se encuentren empobrecidos en su contenido de materia orgánica.

También resultó reseñable el gran descenso de las puntuaciones para el indicador CBM en todos los manejos, respecto a las obtenidas en la profundidad 0-5 cm. Para los tres manejos las puntuaciones fueron cercanas a cero, lo cual indica la baja presencia de microorganismos por debajo de los 5 cm de profundidad en el suelo estudiado.

El índice de calidad de suelo global calculado mediante SMAF fue significativamente diferente entre manejos para la primera profundidad (0-5 cm), pero en la profundidad 5-15 cm no se obtuvieron diferencias. Los resultados mostrados en el capítulo V indican que el mayor índice de calidad general obtenido por NL en la

profundidad 0-5 cm, se debe principalmente a las altas puntuaciones obtenidas por este manejo en cuanto a estabilidad de los agregados, P, COT y CBM. En la profundidad 5-15 cm las puntuaciones obtenidas por los indicadores P y CBM descendieron notablemente, y como consecuencia no se obtuvieron diferencias entre tratamientos en el índice global de calidad del suelo. Estos resultados son comparables a los obtenidos por Karlen et al. (2013), en una evaluación de calidad de suelo realizada mediante SMAF para un ensayo de técnicas de laboreo en Iowa. A pesar de las diferencias en las condiciones edafoclimáticas, también obtuvieron las puntuaciones menores para el laboreo convencional, el cual tuvo el impacto más negativo en varios indicadores de la calidad del suelo. Además, y de acuerdo con los resultados obtenidos en nuestro estudio, la estabilidad de los agregados, COT y CBM fueron los indicadores que mostraron mayores diferencias entre tratamientos.

En cuanto a la metodología de estudio de la calidad de suelo, resulta importante valorar la adecuación del índice utilizado (SMAF) a nuestras condiciones locales. Al observarse los resultados obtenidos en el capítulo V por el suelo estudiado, se comprobó que según SMAF su funcionamiento se encontraba entre el 62 y el 76 % de su potencial para la profundidad 0-5 cm, y entre el 64 y el 65 % en la profundidad 5-15 cm, dependiendo del manejo. Estos resultados son bajos si los comparamos con los obtenidos en estudios previos que han utilizado SMAF. Por ejemplo, Karlen et al. (2006) evaluaron diferentes rotaciones de cultivo en tres localizaciones en los estados de Iowa y Wisconsin, obteniendo puntuaciones mayores de 90, 80 y 75 % en cada caso para la profundidad 0-15. Stott et al. (2011) en un estudio de zonas con diferente desarrollo de la biomasa vegetal de los cultivos a lo largo de la cuenca del río South Fork en Iowa, encontró resultados medios de 86 y 82 % para zonas con vegetación bien desarrollada y escasamente desarrollada en la profundidad 0-10 cm. En estudios que como en el nuestro se distinguió entre distintas profundidades, las puntuaciones obtenidas mediante SMAF para la profundidad 0-5 cm estuvieron siempre por encima del 80 % (Jokela et al., 2009; Karlen et al., 2011; Karlen et al., 2013). En estos estudios también se observa, al igual que en el nuestro, como en todos los

casos las puntuaciones obtenidas para la segunda profundiad 5-15 cm fueron menores (Jokela et al., 2009; Karlen et al., 2013). Las puntuaciones obtenidas (53-69 %) por Karlen et al. (2011) para la profundidad 5-15 cm fueron las únicas en el mismo rango que las obtenidas en nuestro estudio. Todos los estudios mencionados fueron localizados en el medio oeste de los Estados Unidos, por lo que las comparaciones con nuestras condiciones deben ser consideradas cuidadosamente. A pesar de ello, las puntuaciones sistemáticamente bajas en nuestro trabajo, sugieren que aunque SMAF cuenta con flexibilidad para adaptar sus algoritmos a diferentes condiciones edafoclimáticas, es probable que se necesite una calibración de esta herramienta para ser utilizada con mayores garantías en nuestra región. Esta calibración ayudaría a validar las puntuaciones obtenidas mediante SMAF y comprobar si realmente responden a la capacidad del suelo para alcanzar su máxima funcionalidad en el ecosistema en que se encuentra. No obstante, debe ser subrayado que, a pesar de las limitaciones, SMAF ha demostrado ser una herramienta efectiva para la comparación de distintos manejos del suelo en condiciones semiáridas mediterráneas de regadío. Las puntuaciones obtenidas por SMAF para los distintos indicadores han supuesto diferencias significativas entre tratamientos, confirmando su sensibilidad a la hora de detectar diferencias en la calidad de suelo entre manejos agrícolas diferenciados.

Visión global y consideraciones finales

La transformación a regadío de suelos agrícolas supone un gran cambio en las características y funcionamiento del ecosistema agrario. En concreto, la calidad y el funcionamiento del suelo en sí pueden verse modificados. El conocimiento actual, y la experiencia aportada en esta tesis, indican que pueden propiciarse importantes cambios en el ciclo de incorporación y estabilización de la materia orgánica en el suelo. Estos cambios pueden tener efectos significativos tanto en la calidad global del suelo, como en el ciclo del carbono, interviniendo en el reservorio presente en la pedosfera, y en los flujos que se dan entre atmósfera, biosfera y pedosfera.

La colección de estudios llevados a cabo en el marco de esta tesis, ha permitido avanzar en el conocimiento sobre el ciclo de la materia orgánica en suelos carbonatados en la región de clima semiárido mediterráneo y su relación con la dinámica de la agregación y la calidad física del suelo. Además, ha permitido evaluar la interacción de estos ciclos con diversos manejos agrícolas, así como con la transformación a regadío que está teniendo lugar en la región.

Este conocimiento sobre el ciclo de la materia orgánica ha permitido recabar datos sobre las potencialidades del regadío y otros manejos agrícolas, como el no laboreo, para el estabilización del C orgánico (secuestro de C) en suelos agrícolas de la región. Aunque los datos indican una gran potencialidad de estos suelos para la estabilización de carbono orgánico tras la puesta en regadío, es importante destacar la gran cantidad de factores tanto edafoclimáticos como debidos al manejo agrícola que intervienen y de los que dependerá dicha estabilización.

Los trabajos científicos realizados durante la presente tesis también han dado lugar a nuevas cuestiones por resolver y permiten establecer interesantes líneas de actuación a futuro. En primer lugar, el estudio de la dinámica de la materia orgánica a través de herramientas de análisis isotópico, ha demostrado su eficacia para evaluar cambios en el corto plazo derivados de la implantación del regadío. Por ello, continuar con esta línea de trabajo permitiría un estudio más profundo de la dinámica de la materia orgánica, a través de una serie temporal de datos más extensa. Además, la aplicación de dicha herramienta de análisis al estudio comparativo de otros manejos agrícolas que pueden interaccionar con la dinámica de la materia orgánica podría resultar del mismo modo interesante.

El estudio de la calidad del suelo también ofrece líneas de trabajo interesantes en las que profundizar. Desarrollar una metodología reproducible y fiable para su estudio en la región resulta indispensable para ser eficaces en la evaluación de la sostenibilidad de los diferentes sistemas de manejo en relación a su efecto en este recurso. Para ello, y a partir de los resultados obtenidos, trabajar en la aplicabilidad en la región de un índice reconocido

internacionalmente como SMAF resultaría de gran interés. En concreto, profundizar en la adaptación de sus algoritmos de evaluación a las condiciones locales, así como, trabajar en el uso de nuevos indicadores, principalmente biológicos, son dos líneas de trabajo de gran interés.

CONCLUSIONES / CONCLUSIONS

Las conclusiones obtenidas de los estudios que conforman esta tesis son las siguientes:

1. La transformación de secano a regadío en Navarra, monitorizada en diversos suelos tras 5, 7 y 9 años, supuso, de manera general, un incremento en el contenido de C orgánico en el suelo. La magnitud de este cambio varió en función de las condiciones edafoclimáticas y el manejo agrícola. Los factores intrínsecos del suelo (textura) fueron aquellos con mayor relevancia en los suelos monitorizados en esta región.

The transformation of rainfed agricultural soils to irrigation in Navarra, as monitored in different soils after 5, 7 and 9 years, implied a general increment in the amount of organic C stored in the soil. This increment was however different for different soil, climate and management conditions. Soil factors (texture) were the most significant factors modulating this increment.

2. La implantación del regadío en un suelo cultivado en clima mediterráneo, y rico en carbonatos, supuso la **aceleración del ciclo de incorporación de la materia orgánica.** Durante dos años de cultivo de maíz en secano y en regadío la **cantidad de materia orgánica incorporada en regadío, con un 16,58% de C orgánico de origen maíz, dobló a la de secano con un 8, 61%.** El cambio en la dinámica de la materia orgánica propiciado por la implantación del regadío no supuso, en estos dos años, un aumento en el contenido de C orgánico en el suelo. La introducción del regadío en áreas semiáridas de secano puede por lo tanto inducir cambios detectables en la dinámica de la materia orgánica con anterioridad a cambios detectables en el contenido de carbono orgánico, o en la calidad física del suelo. **Las consecuencias potenciales de estos cambios sobre la fertilidad del suelo, la calidad del suelo y el secuestro de**

carbono deben tenerse en cuenta al evaluar el impacto de la transformación a regadío en zonas semiáridas.

The introduction of irrigation in a carbonated Mediterranean soil resulted in faster turnover rates of organic C incorporation. In the first two years, the proportion of maize-derived organic C incorporated to the soil was of 16.58% with irrigation, for 8.61% without irrigation. This was however not reflected in any increment in the amount of organic C stored in the soil. This means that such change in soil management can induce changes in soil organic C dynamics before detectable changes in stocks or the soil physical quality occur. The consequences of this accelerated renovation of organic C on soil fertility, soil physics and organic C sequestration should therefore be evaluated when considering the overall impact of irrigation in semiarid land.

3. La presencia de carbonatos alcalinotérreos en la matriz mineral del suelo tiene consecuencias tanto en su arquitectura como en la cantidad y calidad de materia orgánica que contiene. Por un lado, la composición física de los macroagregados presenta una **distribución volumétrica de los microporos** diferente a los suelos control sin carbonatos. Además, las diferencias observadas en la dinámica de degradación de la materia orgánica entre este tipo de suelos y los controles sin carbonatos evidencian una **mayor protección de la materia orgánica frente a la actividad microbiana**. Sin embargo, **las características físicas observadas en los suelos con carbonatos no parecen ser más limitantes para la descomposición de la materia orgánica, por lo que no pueden explicar por sí mismas las diferencias en labilidad de la materia orgánica**.

The existence of carbonates in the soil mineral matrix has consequences both in the physical architecture of this matrix and on the amount and quality of organic matter stored in it. On one hand, the micropores within macroaggregates showed a different distribution than in similar soils without carbonates. On the other hand, differences observed in organic matter degradation in comparison to non-carbonated soils suggest a greater protection against microbial degradation. However, the physical characteristics observed in carbonate-rich soils seem not to be limiting for organic matter degradation, and cannot explain alone the differences observed in organic matter lability.

4. Las técnicas de análisis térmico han demostrado ser un método adecuado que permite realizar una medición simultánea del carbono orgánico e inorgánico en suelos carbonatados. También permiten evitar el uso de la fumigación ácida, que además presenta dificultades para la eliminación completa de los carbonatos. Además, las muestras sometidas a esta fumigación ácida pueden sufrir cambios en la labilidad térmica de su materia orgánica, los cuales pueden influenciar análisis de calidad de la materia orgánica que se realicen después del tratamiento ácido.

Thermal analysis seems to be an adequate method for the simoultaneous analysis of organic and inorganic C in carbonated soils. This technique also allows avoiding acid fumigation, which rarely achieves complete carbonates removal and affects the thermal characteristics of the organic fraction, which might biass the analysis of organic matter quality following decarbonation.

5. El estudio de **indicadores de calidad de suelo** ha mostrado la necesidad de su r**eevaluación cuando se ven modificadas significativamente las condiciones del agrosistema**, tal y como es el caso en las transformaciones del secano al regadío.

The study of soil quality indicators showed the need for its re-evaluation when the conditions of the agrosystem suffer major changes, such as in the transformation from rainfed to irrigated agriculture.

6. El uso de SMAF ha resultado adecuado para evaluar diferencias de calidad de suelo entre los distintos manejos estudiados, y es sensible a las diferencias observadas entre parámetros de suelo bajo diferentes manejos. A pesar de ello, **se considera necesaria una evaluación detallada de los algoritmos de interpretación de SMAF para comprobar su adaptación a nuestras condiciones agroclimáticas**.

The use of the Soil Management Assessment Framework (SMAF) has been observed to be adequate in the evaluation of soil quality among the soil management systems under irrigation studied in this work. However, a detalied evaluation of the algorithms used for scoring different soil parameters in SMAF is needed for an accurate verification of its potential adaptation to Mediterranean agro-climtaic conditions.

7. La introducción del regadío supone, desde un punto de vista general, cambios significativos en la calidad de los suelos y en la dinámica de la materia orgánica de los mismos. Estos cambios son función de las características edafoclimáticas y de manejo, y deberían considerarse en la evaluación del impacto de esta introducción. En este contexto, es importante avanzar hacia el desarrollo de herramientas sistematizadas que permitan evaluar la calidad del suelo, entendida como su capacidad de funcionar sin degradarse, y su respuesta a cambios en su gestión, adaptadas a las condiciones edafoclimáticas regionales.

The introduction of irrigation induces, from a general perspective, significant changes in organic matter dynamics and soil quality. These changes are modulated by the soil, climate and management characteristics of the agrosystems involved, and should be considered in the general evaluation of the impact of irrigation. In this framework, it seems important to develop systematic tools adapted to local soil and climate conditions, and allowing for soil quality evaluation, and the possible responses of soil to changes in soil management in these circumstances, understanding soil quality as its ability to function without degradation.

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